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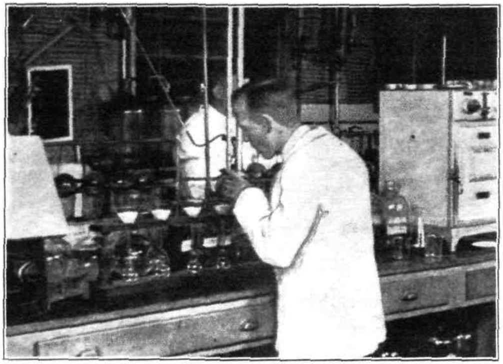

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POULTRY SCIENCE

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Egg Turning, Pipping Position and Malpositions

T. C. Byerly and M. W. Olsen

Bureau of Animal Industry, U. S. Department of Agriculture

(Received for publication April 10, 1937)

THE ability of the chick embryo to orient itself within the shell prior to hatching time is a subject of dispute. Hutt and Pilkey (1934) stated that if eggs are placed horizontally on a tray on the eighteenth day, and not turned subsequently, all eggs which hatch are pipped on the upper surface. They stated further that eggs turned on the nineteenth and twentieth days are pipped about equally on upper, lower, and intermediate surfaces. They presented no data to support their statements.

Waters (1935a) presented data for 225 eggs turned four times daily after the seventeenth day and for 225 controls not turned after the seventeenth day. The turned eggs were pipped about equally on all surfaces, as Hutt and Pilkey stated, but the unturned eggs were also pipped about equally on all surfaces.

Byerly and Olsen (1936) reported that about 5 percent of eggs not turned after the eighteenth day were pipped on the lower side and that the embryos contained by such eggs were frequently in the malposition "head-away-from air-cell."

Data presented in the present communication are for eggs from crossbred flocks, incubated for 13 to 17 days in a forced-draft incubator at 99.5-100°F. and turned twice daily. They were transferred on the fourteenth or eighteenth day to a hatcher at 97.2-97.5°F. for the remainder of the incubation period. Some of the eggs in the hatcher were turned on the nineteenth and twentieth days. The upper surface of each egg was marked after it had been placed in the hatcher so that it was certain that eggs presumed not to be turned were not accidentally turned and eggs intended to be turned could be turned approximately 180 degrees.

The data for pipping position are shown graphically in Figure 1. The data are plotted as a percent of total eggs pipped under each set of experimental conditions. Data were classified into 45 degree classes of the circumference of the egg, viewed at the large end. The center of the zero class was the 9 o'clock position, and the other classes follow in clockwise order.

The eggs turned for the last time on the fourteenth and those last turned on the eighteenth days show a preponderance of pipping in the uppermost 45 degree arc of the shell. Eggs turned over on the nineteenth day were pipped only about twice as frequently on the upper surface as on the

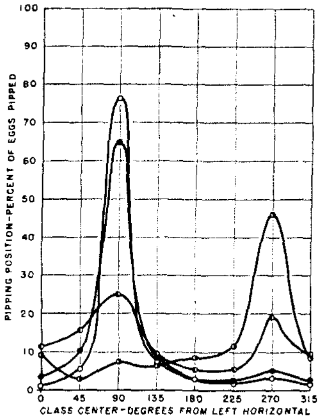

FIG. 1. Pipping position in eggs turned during various portions of the incubation period then incubated in a horizontal position, without turning, to hatching time. ○ Eggs turned for 14 days—3,575 pipped eggs. ● Eggs turned for 18 days—981 eggs. ◐ Eggs turned for 19 days—1,112 eggs. ◑ Eggs turned for 20 days—460 eggs.

lower surface. Forty-five percent of the eggs turned on the twentieth day were pipped in the lowermost 45 degree arc.

Figure 2 portrays graphically the position of pipping in a small lot of control eggs last turned early on the nineteenth day of incubation and the position of the tips of the beaks in a simultaneously incubated lot of eggs last turned on the nineteenth day but immediately chilled. The chilled eggs were not broken until the control eggs had hatched. It was presumed that this experiment would differentiate between self-turning due to embryo activity and passive turning due to the action of gravity on the egg contents.

About three-fourths of the control eggs were pipped on the upper shell surface. About three-fourths of the chilled embryos had their beak-tips near the lower shell surface.

DISCUSSION

Waters (1935a and b) contended that the chick shifts quite frequently after the eighteenth day of incubation. In fact, he concluded that the position head-between-legs is the approximate normal position for nearly all 18-day embryos and that they *subsequently shift to the normal hatching* position. Byerly and Olsen (1936) also presented data which indicated that embryos do sometimes shift from head-between-legs to normal position. The data presented in the present paper indicate a considerable degree of rotation by the embryo in response to gravity. That the chick rotates during hatching is common knowledge.

It would appear that Waters' (1935) data for pipping position are not at all representative. Possibly some of the eggs were shifed by accident or the contained embryos were unusually precocious.

Waters' (1935a) data showing that the position head-between-legs is a normal position on the eighteenth day of incubation is confirmed by the observations of Byerly and Olsen (1936) though Waters observed a higher percentage of such embryos than did the latter authors. The work of Waters is in agreement with that of Byerly and

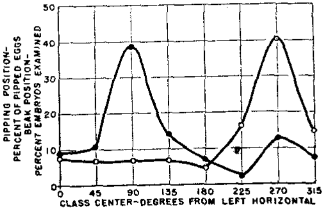

FIG. 2. Pipping position in eggs turned for 19 days, ●—57 pipped eggs. Beak position ○—90 eggs—of embryos in eggs turned on the nineteenth day then chilled to prevent embryo movement.

Olsen in that both sets of data indicate that the malposition head-between-legs is a retardation effect. That is, an earlier normal position persists beyond the normal time and thus becomes a malposition. Waters' conjecture that embryos in the head-between-legs malposition classified as 21-day embryos may have been younger at the time of death is unlikely. An embryo retarded in one respect is not likely to be generally precocious.

If it be granted that head-between-legs should be considered a normal position to the end of the nineteenth day, Waters' (1935a) own data for position of dead embryos on the twenty-second day show that only about 8 percent of the total number of embryos in the malpositions head-between-legs, head-in-small-end, head-to-left and head-away-from-air-cell would be transferred to the normal category. The present authors agree that the malpositions feet-over-head and beak-over-wing are probably too minor aberrations to be separated from the "normal" classification.

SUMMARY AND CONCLUSIONS

Horizontally placed eggs not turned after the eighteenth day of incubation were pipped on the upper shell surface in 90 percent of those pipped.

Horizontally placed eggs turned about their long axes on the nineteenth day of incubation were pipped on all surfaces.

Horizontally placed eggs turned about their long axes on the twentieth day of incubation were pipped on the lower shell surface in about 60 percent of those pipped.

About half the embryos in horizontally placed eggs turned about their long axes on the nineteenth day oriented themselves with respect to gravity through their own efforts.

Literature

Byerly, T. C. and M. W. Olsen, 1936. Certain factors affecting the incidence of malpositions among embryos of the domestic fowl. Poul. Sci. 15:163-168.

Hutt, F. B. and A. M. Pilkey, 1934. Studies in embryonic mortality in the fowl. V. Poul. Sci. 13:3-14.

Waters, N. F., 1935a. Certain so-called malpositions a normal occurrence in the normal development of the chick embryo. Poul. Sci. 14:208-216.

———, 1935b. Changes in the position of chick embryos after the eighteenth day of incubation. Science 82:66-67.

Prevention by Egg Yolk of the Dermatitis in Chicks Due to Egg White[1]

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(Received for publication April 8, 1937)

TULLY and Franke (1934) showed that when dried whole egg was used as 15 percent of a grain ration fed to chicks a "pellagra-like syndrome" was produced. This condition was not prevented by the inclusion of 5 percent of yeast in the ration or 5 percent of yeast plus 3 percent of meat and bone scraps. In about three weeks the syndrome was characterized by scabby sore areas involving the corners of the mouth, which spread to include the area under the lower mandible. At four to five weeks foot lesions appeared with hardening and cracking of the skin of the bottom of the foot and between the toes. A similar condition has been obtained in this laboratory (Lease and Parsons, 1934) in chicks fed a diet containing 30 percent of egg white. This was cured by the inclusion of 8 to 16 percent of liver residue from Lilly liver extract No. 343 in the diet but not by the use of Eli Lilly & Co. liver extract No. 343 in comparable amounts. The response of the rat to liver extract and liver residue is qualitatively identical with that of the chick. Thus it would appear that the factor operative in curing the egg white syndrome is the same in both animals. It has been found by us (Parsons and Lease, 1934), however, that the proportion of egg yolk to egg white comparable to that found in whole egg is curative of the pellagra-like manifestations in the rat due to egg white, although this ratio is near the border line of adequacy. On the other hand, Scheunert and Wagner (1927) as well as others fed raw whole egg to rats and found it to be strongly injurious, producing the same symptoms as did egg white in our laboratory.

In view of these somewhat conflicting results it was decided to investigate the possible differences between the response of the chick and the rat. It seemed doubtful that the factor in egg yolk was different from that in liver but rather that there was a quantitative difference in requirement of the chick and the rat.

EXPERIMENTAL

The plan of the experiment was to put day-old chicks on various proportions of egg white to egg yolk to ascertain, if possible, what proportion of egg yolk to egg white was preventive of the pellagra-like syndrome.

Egg yolk was steamed for an hour, dried and extracted with a commercial solvent[2] several times to remove the greater part of the fat.[3] When cooked egg white was

[1] This work was supported in part by a grant from the Alumni Research Foundation. Published with the permission of the Director of the Agricultural Experiment Station.

[2] Skellysolve B.

[3] This procedure reduced the bulk about one-half. Thus all the percentages of egg yolk are given in equivalents. This allowed the inclusion of as much as 110 percent of egg yolk and was thought to be entirely permissible since the factor potent in curing the pellagra-like manifestations in the rat is not extracted by the usual fat solvents (Lease, 1936).

used, the dried fermented product was reconstituted with water and steamed for 1½ hours.

Tully and Franke's original diets[4] were modified somewhat by inclusion of 3 percent of raw dried peanuts to insure adequate amounts of vitamin B_4. Increases in the concentration of egg white or of egg yolk were made at the expense of equivalent weights of ground oats and corn; the other constituents remained constant.

Day-old chicks were put in separate cages on wire screens and individual food and weight records kept. They were observed at about weekly intervals and careful note taken of their condition. Food and water were renewed daily.

RESULTS AND DISCUSSION

Rations were used containing 5 percent of egg white with extracted egg yolk in amounts equivalent to 10, 55, and 110 percent respectively of the dried egg yolk. The proportion of 5 percent of egg white to 10 percent of egg yolk is comparable to the 15 percent whole egg used by Tully and Franke. With the 10 percent level of egg yolk, symptoms were readily obtained in agreement with the findings of these authors (1934). With the 55 percent level the symptoms were prevented and there was moderately good growth. At the 110 percent level growth was excellent and symptoms were entirely absent (Table 1).

In rations containing 10 percent of egg white, the equivalent of 20 of egg yolk (the proportions of yolk to white found in whole egg) was ineffective in preventing the appearance of the pellagra-like symptoms; the equivalent of 50 percent of egg yolk allowed the appearance of a slight degree of symptoms with moderate growth; at the equivalent of 100 there was very excellent growth and no sign of dermatitis.

It would thus appear that the chick differs from the rat in the proportion of factor

[4] Ground yellow corn 27 percent, wheat bran 15, wheat middlings 15, pulverized whole oats 20, dried whole egg 15, dried buttermilk 5, ground limestone 1, NaCl 1, and cod liver oil 1.

TABLE 1.—*Growth of chicks for first 39 days of life on egg white-egg yolk rations*

Egg white	Egg yolk	Average increase in weight	Comments
percent	percent equivalent	gm.	
5	None	149	Swelling, redness and moderate scaly crust at corners of mouth. Feet with horny scales and thickened, cracked balls.
5	10	150	Heavy crust at corners of mouth and under mandible. Feet rough, thickened, cracked balls. Eyelids red and swollen.
5	55	155	Normal appearance.
5	110	223	Normal appearance.
10	None	112	Crusted, swollen red corners of bill. Balls of feet horny, cracked. Eyelids swollen. Two out of 3 died before the end of the 39 days.
10	20	131	Heavy crust at corners of mouth and under mandible. Neck skin leathery and loss of feathers there. Cracked, thickened skin of feet. Eyelids swollen.
10	50	123	Slight crust and reddening of corners of mouth. Feet slightly horny and cracked. Eyelids slightly swollen.
10	100	357	Normal appearance.

necessary to protect against egg white injury rather than in any essential species difference in reaction to egg white or in curability by the factor in the form of egg yolk. It seems apparent that the results of Tully and Franke are attributable to the fact that they used merely the proportion of egg yolk to egg white found in the whole egg whereas it is necessary to increase the egg yolk to five to ten times the amount of egg white fed before it is protective. Further experiments (Lease, Parsons, and Kelly, 1937) substantiate these results. These experiments, involving the use of a concentrate of the active factor, Extract A,[5] given successfully orally or intraperitoneally in higher amounts for the chick than in the case of the rat, also are in accord with our previous results (Lease and Parsons, 1934).

Glanzmann (1934) reported that dried buttermilk exerted an injurious effect on young rats in his experiments, resulting in "severe protein intoxication." In the experiments reported here buttermilk was used in the diet of the chicks but had not been used in the diets of our rats. Even though this factor of the buttermilk constitutes a variable in the two sets of diets, no such influence needs to be postulated to account for the different behavior of the chick and rat in respect to whole egg since the need of the chick for the potent factor has been shown, in other experiments, to be uniformly so much higher than that of the rat.

That the chick responds to substitution of the raw egg white of the diet by cooked egg white as does the rat was shown by the relief of severe chronic symptoms in a chick by cooking the egg white of the ration.

The experimental results presented by Ringrose and Norris (1936) may reasonably be interpreted to bear out our general hypothesis. It has been shown (Parsons and Kelly, 1933, Parsons, Lease, and Kelly, 1937a, b) that egg white steamed for 1½ hours or autoclaved at 15 pounds pressure for six hours is harmless and allows full recovery from the characteristic nutritional disorder if fed alone in a well supplemented ration but has no curative properties if fed in the same ration together with raw egg white. It is clear that the thoroughly cooked egg white has been rendered harmless by the heating process but has not acquired any potent factor. This harmonizes with our general hypothesis (Parsons, Lease and Kelly, 1937a) that the greater the amount of raw egg white in the ration, the greater the need for the potent factor to secure protection or cure. Apparently Ringrose and Norris (1936) were dealing simultaneously with a simple vitamin deficiency and an induced lack of a factor due to the injurious action of the egg white present in the ration in their experiments.

[5] The preparation has been described by Lease (1936).

SUMMARY

In the prevention of the pellagra-like symptoms due to egg white, the chick requires five to ten times as much egg yolk as the egg white of the diet (dry weight), whereas, in experiments in our laboratory, the rat was protected by the proportions found in whole egg, that is, about twice the weight of egg yolk as of egg white.

The difference between the response of the chick and the rat is due to the higher requirement of the chick for the protective factor in comparison with the rat rather than to any essential qualitative difference.

ACKNOWLEDGMENT

Grateful acknowledgment is made to Miss Helen T. Parsons for suggestion of the problem and advice through its progress, and to Eunice Kelly for her coöperation in the care of the chicks.

REFERENCES

Glanzmann, E., 1934. Experimentelle Untersuchungen über die Nahrungsdefekte der Kuhmilch und verschiedener Milchpräparate. Zeit. für Vitaminforsch. 3:2-27.

Lease, Jane G., Helen T. Parsons, and Eunice Kelly, 1937. A comparison in five types of animals of the effects of dietary egg-white and of a specific factor given orally or parenterally. Biochem. J. (in press).

Lease, Jane G., 1936. Some observations on the nature and the partial isolation of the substance curative of the pellagra-like condition due to dietary egg white. Zeit. für Vitaminforsch. 5:110-117.

Lease, Jane G., and Helen T. Parsons, 1934. The relationship of dermatitis in chicks to lack of vitamin B_2 and to dietary egg white. Biochem. J. 28:2109-2115.

Parsons, Helen T., and Jane G. Lease, 1934. Variations in the potency of certain foodstuffs in the cure of dermatitis induced in rats by dietary egg white. Jour. Nutr. 8:57-67.

Parsons, Helen T., Jane G. Lease, and Eunice Kelly, 1937a. The interrelationship between dietary egg white and the requirement for a protective factor in the cure of the nutritive disorder due to egg white. Biochem. J. 31:424-432.

———, 1937b. The effect of the concentration of dietary egg white on the need for a protective factor given orally or parenterally. Proc. Am. Inst. Nutrition (in press).

Parsons, Helen T., and Eunice Kelly, 1933. The effect of heating egg white on certain characteristic pellagra-like manifestations produced in rats by its dietary use. Am. J. Physiol. 104:150-164.

Ringrose, A. T., and L. C. Norris, 1936. Differentiation between vitamin G and an insoluble factor preventing a pellagra-like syndrome in chicks. Jour. Nutr. 12:535-551.

———, 1936. Differentiation between vitamin G and a soluble factor preventing a pellagra-like syndrome in chicks. Jour. Nutr. 12:553-569.

Scheunert, A., and E. Wagner, 1927. Ueber den Einfluss des Kochens auf den Nährwert des Hühnereies. Deutsche med. Wochnschr. 53:1258-1260.

Tully, W. C., and Kurt W. Franke, 1934. A nutritional disease demonstrating a feed deficiency in dried eggs. Poul. Sci. 8:343-347.

Hemorrhagic Enteritis in Turkeys*

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(Received for publication April 30, 1937)

INTRODUCTION

AN ACUTE hemorrhagic enteritis was found at the autopsy of 35 turkey poults during the months of June, July and August of 1936. The ages of the affected birds varied from 7 to 12 weeks. The specimens originated from widely separated farms in Minnesota. The disease appeared in small flocks consisting of several hundred birds as well as in larger groups containing several thousand or more birds. The size of the flock apparently made no difference as far as the percent of loss was concerned. The mortality averaged approximately 10 percent.

Enteritis in poults is not an uncommon condition. Intestinal disorders may be the result of a large variety of factors: protozoan, bacterial, dietary and possibly climatic. The literature relating to enteric conditions is voluminous. A review of same is not included.

SYMPTOMATOLOGY

In the majority of instances the birds that were examined were found to be well developed and in a good state of nutrition. Occasionally drowsiness, ruffled feathers and a dark discoloration of the head were observed a short time before death. Blood-stained or unnatural droppings were not noticed before death, but following death it was not uncommon to observe a water-like discharge seeping from the vent. Often the first evidence of disease was the finding of one or more dead birds.

POST-MORTEM FINDINGS

The carcasses, with few exceptions, were representative of what one would observe in well nourished and rapidly growing birds. Numerous capillary hemorrhages were found in the breast and thigh muscles. Petechial hemorrhages on the apex of the heart, on the auricular fat and on the auricles were not uncommon. The lungs appeared pale and edematous. Congestion of the lungs was observed in a few cases. Quite often the liver presented no gross lesions, but it was not unusual to find petechial and ecchymotic hemorrhages along the border and upon the surface. Splenomegaly was not found, but a dark discoloration was more or less regularly present. The kidneys were pale, and occasionally a few petechial hemorrhages were observed on the surface. Ecchymoses were present in the mucosa at the junction of the proventriculus and gizzard and in the region of the pyloric opening. These hemorrhages often extended to the serosa. Occasionally the cornified epithelium of the gizzard contained hemorrhages. The entire intestinal tract was distended, extremely cyanotic and filled with sanguineous material. The mucosa was markedly congested. The inflammatory changes were most prominent in the duodenal portion. Other parts of the tract were less seriously involved. Abnormal changes of the caeca were not regularly found. In some cases these appendages were filled with blood, and the mucosa showed evi-

* Paper No. 1504 of the Journal Series of the Minn. Agric. Experiment Station. Completion of this project was made possible by workers supplied on Project No. 1985, Minnesota Works Progress Administration. Sponsor: University of Minnesota.

dence of inflammation. The larynx, trachea, esophagus and crop showed no gross changes.

HISTOPATHOLOGICAL EXAMINATION

The significant pathology was confined principally to the mucosa of the intestinal tract. It was possible to trace the progressive pathological changes by studying tissues obtained from a series of birds. The initial stage consisted of an infiltration of the *lamina propria* with numerous erythrocytes and round cells. (See Fig. 1). The villi very often became distended and resembled club-shaped structures on account of the core becoming filled with cells. (See Fig. 2). The next stage consisted of a separation of the basement membrane from the *lamina propria* and a complete desquamation of the epithelium from the surface of the villi. This left the *lamina propria* extending into the lumen of the intestine, and finally the cores of the villi were eroded or destroyed to such an extent that all that remained in many places was a small tuft extending into the lumen of the intestine. (See Fig. 3). The glands of Lieberkühn, located in the mucosa, did not appear to be involved.

The microscopic changes in the liver varied. In some cases slight congestion was found, in others a considerable degree of hemorrhage was present. (See Fig. 4). Occasionally the changes were more marked and consisted of cellular degeneration and destruction.

The pulmonary lesions consisted of congestion in some cases, and in others, hepatization and induration of the lungs were found.

The renal lesions were the least significant and consisted chiefly of an intertubular hemorrhage.

BACTERIOLOGICAL EXAMINATION

Serum agar slants were streaked with material obtained from the liver, spleen, kidney and heart blood. In the majority of instances the cultures remained sterile at the end of 96 hours of incubation at 37.5°C. temperature. *E. coli communis* was isolated from the liver and heart of a few cases. A gram positive, slender bacillus was also recovered from the heart and liver of a few specimens. This organism grew in dextrose, lactose, sucrose, mannite and maltose, but failed to acidify these carbohydrates. Gas was not produced.

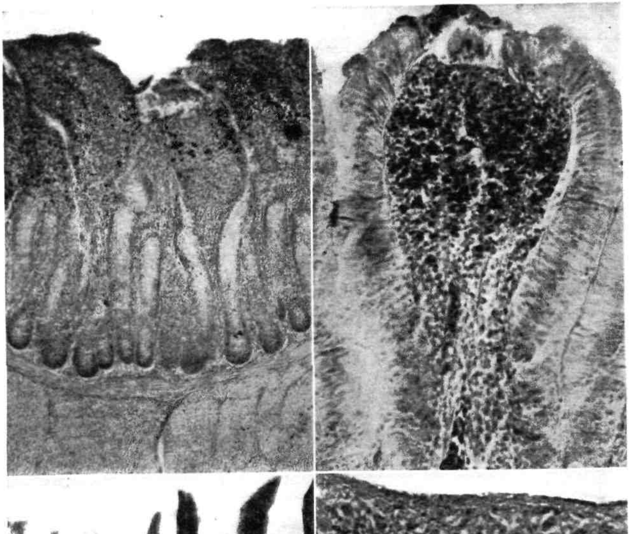
Saline suspensions were prepared by swabbing and scraping the intestinal mucosa. Eosin-methylene blue plates and agar plates were streaked with the saline suspension. By this method *E. coli communis* was isolated regularly. *E. coli communior* and a nonhemolytic staphylococcus were also recovered.

Anerobic cultures were prepared. Liver brain media and deep agar were inoculated with heart blood and liver tissue. *E. coli communis* was isolated in a few instances by means of deep agar cultures whereas no growth appeared on the serum agar slants or in the liver brain media at the end of 96 hours of incubation.

EXPERIMENTAL ANIMAL INOCULATIONS

Normal saline was added to intestinal contents and thoroughly mixed in a mortar. A portion of the suspension was passed through a thin layer of sterile cotton. Guinea pigs, rabbits and turkeys were inoculated subcutaneously with the strained suspension. At the same time, the suspension which had not been strained was fed to a similar series of experimental animals. The latter group remained unaffected. Those that had been inoculated subcutaneously succumbed within 24 to 48 hours. *E. coli communis* was recovered in each instance from the liver and heart.

Intestinal contents suspended in normal saline were passed through a Seitz filter. The resulting filtrate was inoculated subcutaneously into the same species of ani-



(See opposite page for explanation)

mals. These animals remained unaffected.

Heart blood, diluted with normal saline, was inoculated subcutaneously into guinea pigs and rabbits. Negative results were obtained.

Portions of liver, spleen, and kidney were macerated in normal saline solution. This was fed to guinea pigs, rabbits, and turkeys. The oral administration gave negative results. At the same time another similar series of animals was inoculated subcutaneously with the organ suspension. One of two rabbits inoculated died on the seventh day. *E. coli communis* and a gram positive bacillus were recovered from the heart and liver. One of four guinea pigs inoculated died 48 hours later. Organisms similar to those obtained from the rabbit were recovered. Two of four turkeys inoculated died 96 hours later. *E. coli communior* was recovered from the internal organs.

A saline suspension was prepared from a culture of the gram positive organism. One rabbit and one guinea pig were inoculated subcutaneously with the culture. The rabbit died within 24 hours and the guinea pig died on the 15th day. The guinea pig was found to have pneumonia, and *E. coli communis* was isolated from the heart and liver. Another series of guinea pigs and rabbits was inoculated with a 24 hour transfer obtained from a fortnight-old culture of the gram positive bacillus suspended in saline solution. In this instance negative results were obtained.

Unless otherwise specified, two rabbits, four guinea pigs and four turkeys were used in each of the above experimental procedures.

HEMATOLOGICAL EXAMINATION

The blood of two sick turkeys was examined. The following is a summary of the findings: No. 1. Hemoglobin (Dare) 30 percent, total erythrocytes 1,000.000, total leucocytes 15,550. Differential leucocyte count: lymphocytes 51 percent, polymorphonuclear 46 percent and basophils 3 percent. No. 2. Hemoglobin (Dare) 40 percent, total erythrocytes, 1,470,000, total leucocytes 20,660. Differential leucocyte count: lymphocytes 22 percent, polymorphonuclears 76 percent, and basophils 2 percent.

Blood smear examinations revealed polychromasia and hypochromasia. Some of the erythrocytes were found to show a slight degree of immaturity. The latter has been interpreted as a normal process of blood regeneration brought about by the intestinal hemorrhage.

SEROLOGICAL EXAMINATION

Twelve turkeys which had survived the experimental inoculations described before were used in this part of the study. Prior to inoculation all birds were found negative to the rapid whole blood agglutination test for pullorum disease. Two turkeys had been inoculated subcutaneously with a Seitz filtrate obtained from the intestinal contents, two were similarly inoculated with an organ suspension, three received per os unfiltered intestinal contents, and five were fed portions of macerated organs suspended in normal saline.

Test tube antigens were prepared by using the following organisms: *S. aertycke* of turkey origin, *S. pullorum* of avian origin, *S. gallinarum* of avian origin, *S. paratypho-*

Fig. 1. 70X. A photomicrograph of the duodenum showing an infiltration of the *lamina propria* with erythrocytes and round cells. An early stage.

Fig. 2. 290X. Photomicrograph, the same as Fig. 1 with higher magnification.

Fig. 3. 70X. Photomicrograph showing terminal stage. Note that many of the villi have been destroyed, others have only the core remaining.

Fig. 4. 120X. Photomicrograph of liver showing areas of hemorrhage.

sus A of human origin, *S. paratyphosus* B of human origin, *S. suipestifer* of porcine origin, *S. enteritidis* of rat origin, and *S. abortivo-equina* of equine origin. The blood serums of the turkeys were each tested with the above antigens at two and four-week intervals following inoculation. The following dilutions were used: 1:25, 1:50, 1:100, 1:250, 1:500, and 1:1000. None of the blood serums were found to contain agglutinins for these antigens.

DISCUSSION

According to the information obtained from the owners of affected flocks, the young turkeys had been reared, until they were six to eight weeks old, on porches equipped with wire screens. They were then placed on field ranges. There were no unusual losses until the birds had been on the outside yards for ten to fourteen days. Apparently no deaths occurred as a result of this condition while the birds were confined to the wire screened porches.

During the period when the losses were most severe, the temperature and moisture reports of the Minneapolis weather bureau indicated a marked variation from that of an average year. A very dry and hot period was experienced. The following are the mean temperatures during the average year for the months of June, July and August respectively: 67.8°, 72.7° and 69.6°. The mean temperatures during 1936 for the same months were respectively: 66.4°, 81.4° and 74.7°. The rainfall (in inches) during an average year for June is 4.7, July, 3.41, and August 3.36. In 1936 the precipitation was materially lower: June 2.29 inches, July 0.11 inches, and August 0.348 inches. As a result of this dry, hot period, the range conditions were not satisfactory. The soil became parched, and greens were not available in sufficient quantity. At the same time the owners had not provided proper shelter, and the birds had no protection during the heat of the day.

It is known that the intestinal flora of normal chickens contains colon organisms similar to those recovered in this study.

Emmel (1) reported the isolation of typhosus, paratyphosus A, paratyphosus B, suipestifer, aertrycke and enteritidis from the intestinal tract of chickens.

The pathogenicity of *E. coli* for guinea pigs and rabbits varies with different strains. Those isolated from the healthy intestine are usually not as virulent as those derived from an inflamed bowel (2).

Beaudette and Hudson (3) recovered an organism from turkeys that satisfied the morphological, cultural and biochemic requirements of the true swine erysipelas organism. In their cases large or small blotch hemorrhages were found in the muscles over the posterior ribs, and in a few cases, subpleural hemorrhages occurred on the breastbone. These authors described other post-mortem findings that somewhat resemble those that we found. However, we regularly found the intestines filled with a sanguineous material. We were not able to recover *Erysipelothrix rhusiopathiae*.

The organisms recovered in this study have not been considered of primary importance as far as etiology is concerned. Secondarily, they might have been of consequence.

Provision of additional shelter and range, supplemented with an abundance of cold drinking water and milk, was advised. The losses subsided as the season progressed and the temperature became more moderate.

BIBLIOGRAPHY

Beaudette, F. R. and Hudson, C. B., 1936. An outbreak of acute swine erysipelas infection in turkeys. Jour. A.V.M.A. Vol. LXXXVIII, N.S. April, 4:475-487.

Bulloch, W., 1929. A system of bacteriology. Medical Research Council. 4:266-283.

Emmel, M. W., 1936. The intestinal flora of chickens affected with enteritis associated with intestinal parasites. University of Florida Experiment Station. Bulletin 293.

The Vitamin A Content of Pilchard Oil

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WITHIN the past few years there has developed an increasing interest in the use of pilchard (sardine) oil for poultry feeding, particularly on the West Coast, where its price is considerably lower than that of good cod liver oil. Poultry keepers in British Columbia and in California claim that, at the ordinary levels of feeding, pilchard oil gives as satisfactory growth as cod liver oil. These claims are apparently supported by the results of feeding trials conducted by Gutteridge (1933).

Spectrophotometric essays by Shrum and How (1935) and by McFarlane and Rudolph (1936) show pilchard oils to be low in vitamin A (160-350 I.U. per gram) as compared with the better quality cod liver oils of poultry grade (700-1500 I.U. per gram). It is evident that in order to reconcile the high growth rates reported for chicks fed pilchard oil with the low spectrophotometric assays of this oil, further critical study must be made of the methods of assay.

Pilchard oil, as is well known, has a characteristic deep golden brown color as compared with the clear yellow of cod liver oil. It has been suggested that pilchard oil may have a high content of carotinoid pigments, and that these pigments may have growth-promoting value for the chick and thus account for the alleged difference between the spectrographic and the biological assay.

In the experiments recorded below this possibility has been investigated.

EXPERIMENTAL

PART 1. THE SPECTROSCOPIC DETERMINATION OF THE CAROTINOID PIGMENT AND VITAMIN A CONTENT OF PILCHARD OIL

The procedure outlined below is based upon the observation of Wolff, Overhoff and Eckelen (1930) that carotene can be separated from vitamin A by a phase separation between petrol-ether and 90 percent methyl alcohol.

Pilchard oil (372 gm.) was saponified by boiling with alcoholic potassium hydroxide in an atmosphere of nitrogen. The hydrolysate was repeatedly extracted with ether until the ether extract was colorless. The greater portion of the pigment in pilchard oil is insoluable in ether. The ether extract (5 liters) was concentrated to 250 ml. An aliquot portion was evaporated in-vacuo and the residue dissolved in 10 ml. of absolute alcohol. The vitamin A content of this solution as determined by the spectrophotometer[1] was equivalent to 249 international units per gram of oil.

The entire ether extract was taken to dryness in-vacuo and the residue dissolved in 250 ml. of petrol ether. The petrol-ether solution was then extracted five times by shaking in a separatory funnel with 200 ml. portions of 90 percent methyl alcohol.

1. *Methyl Alcohol Phase*

(a) *Vitamin A*—The absorption value

$$E \begin{cases} 1\,\% \\ 1\text{ cm.} \\ 328\text{ m}\mu \end{cases}$$

in absolute alcohol was equivalent to 182 I.U. of vitamin A per gram of oil.

(b) *Pigment*—The carotinoid pigment in this fraction was not identified. Biely

[1] A Bellingham and Stanley quartz spectograph fitted with a rotating sector was employed.

and Chalmers (1936) believe the pigment to be fucoxanthin. We have made a rough determination of the pigment content of this fraction as Xanthophyll (absorption maxima 455 m μ.). To get a measurable absorption at 455 m μ. a concentrated solution had to be used, with the result that the intense absorption of vitamin A masked the absorption due to the pigment. An indication of the pigment content of this fraction (at least the maximum amount) was obtained by measuring the intensity of absorption at 455 m μ. The extinction coefficient $E \begin{cases} 1\% \\ 1 \text{ cm.} \\ 455 \text{ m}\mu \end{cases}$ of Xanthophyll is 1,570. Using this factor the Xanthophyll content of one gram of the pilchard oil was calculated to no greater than 19 γ. Crediting the pigment with the provitamin A activity of carotene the vitamin A value of the pigment in this fraction might be of the order of 19 international units.

2. Petrol-ether Phase—Carotene

With this fraction, as with the methyl alcohol fraction, a selective absorption band for the pigment was not obtained. Some substance was present which absorbed strongly in the region of 400 m μ. This substance was not extracted by 90 percent methyl alcohol. The extinction-coefficient at 448 m μ was taken as a measure of the upper limit of the carotene content of this fraction. Crystalline mixed carotene[2] in ether solution was found by us to have an extinction coefficient $E \begin{cases} 1\% \\ 1 \text{ cm.} \\ 448 \text{ m}\mu \end{cases}$ of 2,180. Employing this factor, the carotene content of 1 gm. of the pilchard oil was calculated to be of the order of 0.76 γ which is roughly equivalent to one international unit of vitamin A. Summarizing these results, the possible vitamin A equivalent of the pilchard oil would be 182 + 19 + 1 or 202 international units per gram.

[2] S. M. A. Corporation, Cleveland, Ohio.

The efficiency of the extraction of vitamin A from petrol-ether solution by five extractions with an equal volume of 90 percent methyl acohol was tested on the unsaponifiable fraction of two samples of cod liver oil of poultry grade, with the following results:

TABLE 1

	Vitamin A (I.U. per gram of oil*)	
	I	II
Unsaponifiable fraction	1,280	1,110
Methyl alcohol phase		
(a) First 3 extracts combined	729	—
(b) Last 2 extracts combined	168	—
(c) Combined 5 extractions	—	692
Petrol-ether phase	86	42

* Extinction coefficient converted to international units of vitamin A by multiplying by 1,600.

The results in Table 1 show an apparent decrease in vitamin A units of 23.2 percent (oil No. I) and 24.8 percent (oil No. II) in the two phases as compared with the original unsaponifiable fraction. We have already drawn attention (1936) to the fact that a poorly defined absorption band at 328 m μ is obtained even with the unsaponifiable fraction of crude cod liver oils and pilchard oils, and that the vitamin A estimations give results which are too high. Of the total vitamin A units in the combined methyl alcohol and petrol-ether phases, 91 percent (oil No. I) and 94 percent (oil No. II) were contained in the methyl alcohol extract. Since the completion of this work Gillam and Senior (1936) have found that seven extractions of a petroleum-ether solution of vitamin A, with an equal volume of 90 percent methyl alcohol each time, are necessary to extract all the vitamin A.

From absorption measurments before and

TABLE 2
Series 1

B.R. Chicks, ♂ and ♀	Feb. 5 to Mar. 25, 1936			
Control Diet	Pilchard Oil		Cod Liver Oil	
	(Untreated 400 I.U.) (Unsap. frac. 260 I.U.)		(Untreated 1,100 I.U.) (Unsap. frac. 1,018 I.U.)	
Gr. White Rice 60 Wheat Middlings 15 Skim Milk Powder 5 Meat Scrap 10 Yeast (Non-irrad.) 5 Irrad. Yeast .5 $CaCO_3$ 1.5 Salt (NaCl) 1.0 Olive Oil 2.0 100	Pen 2	Pen 3	Pen 4	Pen 5
	Test Oil .50 Olive Oil 1.50	.25 1.75	.50 1.50	.25 1.75

after oxidation of the oil at 90°C., Shrum and How (1935) calculated the vitamin A content of a sample of pilchard oil to be 160 I.U. per gram. Two samples of pilchard oil were assayed on rats by Bailey (1935) and found to contain 120 and 175 I.U. per gram. These results are in good agreement with the vitamin A content of a pilchard oil (182 I.U. per gram) as calculated by us from the absorption measurements at 328 m μ on the 90 percent methyl alcohol soluble fraction of the unsaponifiable material.

PART 2. THE BIOLOGICAL DETERMINATION OF THE VITAMIN A CONTENT OF PILCHARD OIL

Since it is generally accepted that the spectrophotometric method of analysis forms a reliable test of the Vitamin A content of high-potency cod liver oils, comparison of the growth-promoting effect of pilchard oil for chicks was tested against that of high quality cod liver oil on a unit-for-unit basis, both oils being subjected to spectrophotometirc analysis at the time of the experiments. In this way, the biological assay of pilchard oil could be related to the physical test, and a check made on the reliability of spectroscopic tests for vitamin A on these lower potency oils.

Table 2 shows the diets used and the general scheme of the preliminary test, and Table 3 the average weights of the chicks at seven weeks.

The weights of the control pen are not given, as all the chicks had died before the end of the test period. It will be noted that, in spite of the wide difference in vitamin A content of the oils, the weights of the pen on pilchard oil at both levels are not significantly different from those on cod liver oil. These results, which are similar to the findings of Gutteridge (1933), may

TABLE 3
Series 1
Ave. Weights, 7 Weeks

Pen	No. of Chicks	♂	No. of Chicks	♀
2 .5% Pilchard oil	10	507.7	5	426.6
3 .25% Pilchard oil	6	516.7	10	395.7
4 .5% Cod liver oil	9	503.8	6	442.0
5 .25% Cod liver oil	11	518.4	5	461.0

TABLE 4
Series 2

B.R. ♂ Chicks Control Diet		May 20 to July 15, 1936 Revised June 10
Ground White Rice	59	54
Wheat Middlings	20	25
Skim Milk Powder	5	5
Meat Scrap	10	10
Salt (NaCl)	1	1
Irrad. Yeast	1	2
$CaCO_3$	1	1
$Ca_3(PO_4)_2$	1	—
Cottonseed Oil	2	2
	100	100

Cod Liver Oil Pen: (2,000 I.U.)	Cottonseed Oil Cod Liver Oil	1.944 0.056
Pilchard Oil Pen: (450 I.U.)	Cottonseed Oil Pilchard Oil	1.750 0.250

appear to suggest that a pilchard oil of 400 I.U. is equal in biological value to a cod liver oil whose potency is estimated at 1,100 I.U. by physical measurements. It will be noted, however, that the average weights are up to or above normal in all pens, and are as high on the .25 percent as on the .50 percent levels. The amounts of oil fed are thus probably in excess of minimum requirements, and any difference in the growth-promoting value of the two oils is masked.

Tabel 4 gives the diets and the scheme of a test with a second group of chicks (Barred Rock males) in which the levels of oil in the test pens were adjusted so that the total number of units fed (as determined on the untreated samples by the spectrophotometer) was the same in each pen, namely, 112 units per 100 grams of feed, or 0.25 percent of pilchard oil assaying 450 I.U. and 0.056 percent of 2,000-unit cod liver oil.

Table 5 shows growth rates, weekly, for eight weeks, and the number of chicks surviving in each pen. The drop in number of chicks in pens 2 and 3, which was carried back to the second week, is due to the development in each pen of a few cases of Perosis, and to the presence of a few females which were removed when their sex was recognized. In the control pen, individual chicks were removed when vitamin A depletion reached the stage of tremors or paralysis, so that the chicks were unable to eat.

It will be noted that a difference in weight in favor of the pen on 0.056 percent cod liver oil appears at the second week, and increases steadily up to the eighth week. At this time the average difference has become highly significant (*t* test—Fisher 1934). This difference is approximately of the same magnitude as that found to exist between the assay on the untreated pilchard oil (450 I.U.) and that on the unsaponifiable fraction (284 I.U.), indicating that the

TABLE 5
Series 2
Ave. Weights to 8 Weeks, B.R. Male Chicks

Age	Control		0.056% C.L.O. 2,000 I.U.		0.25% P.O. 450 I.U.	
	No.	Wt.	No.	Wt.	No.	Wt.
1 wk.	30	52.6	30	52.9	30	52.8
2 "	28	85.6	23	89.7	23	86.3
3 "	26	142.2	23	154.9	23	143.4
4 "	16	198.2	23	226.7	23	205.0
5 "	—	—	23	323.2	23	289.2
6 "	—	—	23	439.0	23	367.7
7 "	—	—	23	544.3	23	441.8
8 "	—	—	23	649.3	23	485.4

latter figure is a more accurate estimate of the biological value of the oil (with the cod liver oils, there was practically no difference in the two assays). Assuming that rate of growth, other factors being equal, is a function of the vitamin A content of the feed, it would thus require 0.396 percent of this pilchard oil, instead of 0.25 percent as fed, to give a rate of growth equivalent to that on 0.056 percent of the cod liver oil used.

CONCLUSIONS

It is apparent from these tests that although at the higher levels of feeding little difference in value appears as between good pilchard oil and a good grade of cod liver oil, the actual difference in biological value may be quite marked when minimum requirements are fed. In this series of tests the growth rate follows fairly closely the vitamin A assay by means of spectrophotometry.

The spectrophotometric measurement of vitamin A in the unsaponifiable fraction of pilchard oil (even making the fullest allowance for the biological activity of the carotinoid pigments) is thus shown to be a reasonably accurate measurement of the growth-promoting value of the oil, and there is no evidence that it contains, in any significant amount, a growth-promoting factor which is not vitamin A. In spite of the difference in biological value between the two types of oils, however, we would not imply that pilchard oils, as now used by poultry breeders, do not serve a highly useful purpose.

REFERENCES

Bailey, B. E., 1935. Unpublished data. Prince Rupert Fish. Exp. Sta., Biol. Board of Canada.

Biely, J. and Chalmers, W., 1936. Abstracts of the American Poultry Science Association—Annual Meeting.

Fisher, R. A., 1934. Statistical Methods for Research Workers. Oliver and Boyd, Edinburgh.

Gillam, A.E. and Senior, B. J., 1936. Biochem. J. 30:1249.

Gutteridge, H. S., 1933. Sci. Agric. 13:374.

McFarlane, W. D., and Rudolph, L., 1936. Sci. Agric. 16:398.

Shrum, G. M., and How, T. C., 1935. Can. J. Research 13:93.

Wolff, L. K., Overhoff, J., and Eckelen, M., 1930. Dtsch. med. Wschr. 56.

Observations on Some Symptoms of Vitamin A Deficiency in Chicks

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SEVERAL investigators have reported a staggering gait, or "ataxia," as one of the characteristic symptoms of vitamin A deficiency in chicks. Frohring and Wyeno (1934) state that practically all of the chicks they used showed marked ataxia from 3 to 14 days before complete depletion of vitamin A and death, and suggest that the first signs of ataxia would be a safer end point to begin giving the curative dose if chicks are to be used for quantitative tests of vitamin A after depletion. Elvehjem and Neu (1932) describe a staggering gait and a general incoördination of movements among other symptoms of vitamin A deficiency, and state that all chicks placed on their diet developed all of the specific symptoms they described.

EXPERIMENTAL PROCEDURE

Observations were made of the bursa of Fabricius in a total of 536 chicks, of ataxia in a total of 226 chicks, and of retention cysts in a total of 230 chicks in connection with feeding tests of alfalfa leaf meals at the Southwest Poultry Experiment Station, Glendale, Arizona. Five similar trials were conducted, and in each trial one group of chicks was fed the following basal diet

	Percent
Ground white corn	64
Wheat middlings	15
Commercial casein	10
Pure dried yeast	4
Ground limestone	3
Steamed bone meal	1
Salt	1
Viosterol solution 50D	2
Total	100

known to be deficient in vitamin A but adequate in all the other known nutritive factors (Heywang and Titus, 1937).

The chicks received ample direct sunshine in addition to viosterol solution 50D, and there was no known possibility that the chicks received an insuffiicient quantity of the antirachitic factor. With the exception of the dried yeast and the viosterol solution, all of the ingredients were obtained before the tests were started. The dried yeast was purchased in 100-pound lots, and the viosterol solution 750D was purchased in liter quantities and diluted with corn oil to 50D strength prior to mixing with the mash.

Trial 1 was started November 16, 1934; trial 2 on February 8, 1935; trial 3 on May 27, 1935; trial 4 on December 27, 1935; and trial 5 on October 29, 1936. The chicks were purchased from commercial hatcheries, and the vitamin A content of the ration fed the parent stock is not known.

ATAXIA AND VITAMIN A DEFICIENCY

The data in Tables 1 and 2 illustrate the variability among the chicks with regard to the age at which ataxia started and the average length of life afterwards. The variability of the age at which ataxia started may have been due to some extent to the different amounts of vitamin A in the diets of the female parents and the resulting different amounts of vitamin A stored in the chicks when they were hatched. It seems logical to assume that a large part of the stored vitamin A in chicks would, at hatching time, be in the unabsorbed yolk material. Hence,

TABLE 1.—*Variation in age of chicks when ataxia appeared and subsequent days lived*

Age of chicks when ataxia started	Subsequent days lived by individual chicks					
Days	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Av. all trials
11	—	—	—	—	2	2.0
12	—	—	—	3	—	3.0
14	—	—	—	1-5-6	—	4.0
15	—	—	—	1-1-3-9	2-6	3.7
16	2-3-5	—	—	2-2-4-4-4-6-7-7	—	4.2
17	—	6	3-3-7	1-2-3-3-4-5	—	3.7
18	—	—	—	2-4-4-6	6	4.4
19	—	9-10	7-8	3-6-7-9-10	2-2-6	6.6
20	2-4	5-9-12	7-11	1	—	6.4
21	9	—	7	2-3	9	6.0
22	6-8	—	8-17	—	—	9.8
23	7-8-9	11	3-5-6-10	8	4-5-5	6.8
24	3-5-10	7-8	5-6-7	1-9	7-7-12	6.7
25	5-6-8-9	8-9-18	4-6-9-11	3	8-8	8.0
26	3-6	16	6	3	2	6.0
27	6-6-7-7	3-9-21	1-6-7	7	3-5	6.8
28	5-6	—	4-7-14	9	2-5	6.5
29	5	—	12	—	4-5-8	6.8
30	4-12	5-7-8-10-15-22	—	—	3-3-5-10	8.7
31	3-3-9-10	3-7-12-13	—	—	7-8-9	7.6
32	—	5	—	—	—	5.0
33	10	—	2-15	—	2-3	6.4
34	3	—	—	—	—	3.0
35	6	2	—	—	3-3-5	3.8
36	—	10	—	—	—	10.0
37	—	15	—	5	—	10.0
39	—	22	8-10	—	—	13.3
43	—	25	—	—	—	25.0

if chicks were to be used in vitamin A assay studies and the first appearance of ataxia used as the point at which to begin the curative dose, it might be advantageous for uniform, coprehensive results to use only chicks from parent stock fed a ration of uniform vitamin A content and to deutectomize the chicks soon after they were hatched.

Since vitamin A is necessary for growth, the growth curves shown in Figure 1 indicate that the appearance of ataxia is not coincident with the point at which the vitamin A intake is at the level necessary for maintenance of life without increase in body weight. This is clearly shown by the differences among chicks with regard to the gain or loss in body weight, as well as length of life, after ataxia was first apparent. All twelve of the chicks were used

TABLE 2.—*Age of chicks when ataxia appeared and subsequent days lived*

Trial No.	Total No. Chicks	Chicks with ataxia	Av. age when ataxia first appeared	Range of age when ataxia first appeared	Av. duration of life after ataxia first appeared	Range of duration of life after ataxia first appeared
		Number	Days	Days	Days	Days
1	40	36	25.7	16–35	6.1	2–12
2	43	32	27.9	17–43	10.7	2–25
3	47	33	24.9	17–39	7.3	1–17
4	47	42	18.7	12–37	4.4	1–10
5	49	36	26.0	11–35	5.2	2–12

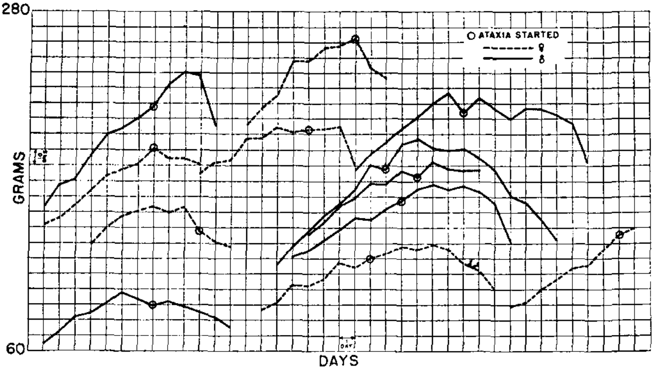

FIG. 1. Weight curves from daily weighings of 12 White Leghorn chicks from seven days previous to the appearance of ataxia until death.

in trial 5, and all had a deposit in the bursa of Fabricius at death.

THE BURSA OF FABRICIUS AND VITAMIN A DEFICIENCY

Hinshaw and Lloyd (1934) mention the bursa of Fabricius as an organ affected in vitamin A deficiency of young birds and describe desposits found in it.

The bursa of Fabricius was examined in every chick in the groups fed the vitamin A deficient diet, and the pertinent data are listed in Table 3. In most cases the deposit was white, and varied in quantity from a few flakes to a plug which distended the bursa of Fabricius to more than normal size. A thick mucoid exudate was noted in very few instances.

All of the chicks that died with a deposit in the bursa of Fabricius had also showed ataxia; no deposit was found in any of the chicks that died without showing ataxia.

That there was a considerable difference among chicks is shown by a comparison of the range in number of days lived for chicks that had a deposit and those that did not. For instance, in trial 5 one chick that lived

TABLE 3.—*Age at which chicks with ataxia died with, and without, deposit in bursa of Fabricius*
Trials 1–5 Basal Diet

Trial no.	With deposit			Without deposit		
	No. chicks	Av. age	Range of age	No. chicks	Av. age	Range of age
		Days	Days		Days	Days
1	20	35.1	29–43	16	27.5	19–34
2	20	42.9	32–68	12	31.5	23–38
3*	21	34.3	26–48	9	28.4	24–31
4	14	28.2	20–42	28	20.6	15–33
5	20	33.3	21–40	16	28.4	13–39

* 3 chicks not examined.

21 days had a deposit while one that lived 39 days did not.

However, in all trials the average age of the chicks that died with the bursa of Fabricius affected was greater than that of the chicks that died without it being affected. This is shown in Table 4 which lists results with chicks fed other diets in the same series of trials. The diets contained some vitamin A, but an insufficient amount to support life during the experimental period. It is thus indicated that a deposit in the bursa of Fabricius would be most likely to appear as an indication of vitamin A deficiency when chicks had received only enough of the vitamin to permit them to live for fairly long periods.

In connection with the data in Tables 2, 3, and 4 it seems pertinent to note that 275, or 52 percent, of the 533 chicks examined had a deposit in the bursa of Fabricius. That percentage is considerably lower than that observed by Hinshaw and Lloyd (loc. cit.) but our data are for nearly twelve times as many chicks. It is also possible that we failed to note a mucoid exudate in some instances where it was present.

RETENTION CYSTS

No mention is found in the available literature of the occurrence of rentention cysts in connection with vitamin A deficiency of chicks. A retention cyst filled with a clear fluid and apparently originating from the ureters before they empty into the bursa of Fabricius was found in approximately 30 percent of a total of 230 chicks that died when fed diets known to be deficient in vitamin A. The cysts were of various sizes, and only one was found in a chick. In Figure 2 is shown a large retention cyst in a chick that lived 48 days on a vitamin A deficient diet. A deposit was found in the bursa of Fabricius in 64 out of 72 chicks that had a retention cyst.

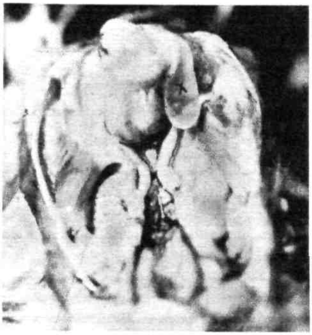

FIG. 2. A large retention cyst in a chick that died from avitaminosis A when 48 days old.

SUMMARY

Chicks fed the same diet varied greatly in the age at which the ataxia caused by

TABLE 4.—*Age at which chicks died with, and without, deposit in bursa of Fabricius*
Other diets

Diet	Trial No.	With deposit			Without deposit		
		No. chicks	Av. age	Range of age	No. chicks	Av. age	Range of age
			Days	Days		Days	Days
A	4	18	35.3	22–52	33	23.3	7–39
A	5	27	35.8	26–46	23	25.5	7–39
B	4	24	53.6	41–67	15	33.0	12–68
B	5	32	52.0	38–66	11	34.0	26–43
C	4	13	41.1	24–70	15	28.9	18–47
C	5	31	42.3	30–59	19	21.7	8–32
D	5	35	51.0	26–68	14	31.1	7–52

vitamin A deficiency appeared, and the number of days lived thereafter. The appearance of ataxia did not indicate that only enough vitamin A for the maintenance of life was available, for some chicks showed an increase in body weight after ataxia was evident.

A deposit was found in the bursa of Fabricius in approximately 52 percent of the chicks examined for evidences of death from vitamin A deficiency. All chicks that had a deposit had previously shown ataxia.

A retention cyst apparently originating from the ureters was found in 72 out of 230 chicks that died when fed vitamin A deficient rations. A deposit in the bursa of Fabricius was also present in all but eight of the chicks with a retention cyst.

REFERENCES

Elvehjem, C. A. and V. F. Neu, 1932. Studies in vitamin A deficiency in the chick. J. Biol. Chem. 97:71-82.

Frohring, W. O. and J. Wyeno, 1934. Carotene and vitamin A requirements for White Leghorn chicks. Jour. Nutr. 8:463-476.

Heywang, B. W. and H. W. Titus, 1937. Alfalfa leaf meal as a source of vitamin A for growing chicks. Jour. Agr. Research.

Hinshaw, W. R. and W. E. Lloyd, 1934. Vitamin A deficiency in turkeys. Hilgardia 8:281-304.

The Effect of Iritis of Breeding Hens on Their Progeny

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(Received for publication May 17, 1937)

THE disease now known as lymphomatosis and its connection with a contracted, non-responsive pupil and discolored iris (iritis) were early mentioned by Kaupp (1921), Doyle (1926), and Pappenheimer et al (1926). Considerable research work has been reported on the etiology of the disease during the past few years, and theories on the method of its dissemination have been advanced. Doyle (1928) believed the disease to be infectious and also believed it quite probable that paralysis may be transmitted from parent to offspring in much the same way as Pullorum disease. The latter conclusion was advanced after studying the offspring from 30 pullets obtained from a flock where heavy loss from paralysis occurred. A high incidence of the disease was present in the offspring and no paralysis or iritis developed in the controls.

Gildow, Williams, and Lampman (1936) found that paralysis was transmitted to chicks by pen contact and that the incidence of the disease was greater in progeny from clean stock than it was in progeny from affected stock, when such chicks were reared together. Approximately 25 percent of the cases of the disease which developed were of the "eye lesion" type. Nerve and eye lesions were most common, tumors and leg paralysis next in prevalence, and general and wing paralysis least prevalent.

Patterson, Wilcke, Murray, and Henderson (1932) found that the disease could be transmitted by injection of lesion suspensions and by pen contact. Almost any conceivable combination in regard to location of lesions, tissues involved, and pathologic types were found by these authors. There was some evidence presented that the disease may be transmitted through the egg, although it was the belief of these workers that the majority of affected birds contract the disease from pen contact. Approximately 50 percent of the progeny from a badly infected flock developed the disease in some form over a period of two years. The progeny from the above affected pullets also showed about the same percentage involvement at approximately one year of age. Control groups showed a considerably lower incidence of the disease.

Johnson (1932) reports that transmission through the egg can hardly be considered tenable but some weakness or susceptibility to the exciting agent might be transmitted from mother to offspring.

Biely and Palmer (1932) conclude that there is an inheritance of resistance to paralysis which depends upon a dominant gene. Therefore, the results obtained in any given transmission experiment would depend upon the genetic constitution of the birds studied.

Dalling and Warrack (1936) make conclusions on their breeding experiments. They are in part as follows:

(1) Incubation and rearing play no part

[1] The author acknowledges helpful suggestions and assistance from Byron Alder, Station Poultryman, in connection with this investigation. Authorized by the director.

in the causation of lymphomatosis.

(2) *Groups of pullets selected for apparent "susceptibility" and "resistance" to lymphomatosis failed to furnish clear evidence of these qualities in their progeny.*

(3) Individual pullets seem to have a considerable amount of influence in the fate of their progeny.

(4) Pullets in whose ancestors lymphomatosis has existed to a marked degree do not necessarily produce affected progeny.

(5) Pullets in whose ancestors there is no evidence of lymphomatosis may produce progeny in which the condition occurs to a marked extent.

(6) Infected dams do not always produce affected progeny.

(7) Sister pullets ("resistant" or "susceptible") do not necessarily all behave alike in the production of affected or healthy progeny.

(8) Mating of father and daughter or brother and sister is not followed by a greater incidence of lymphomatosis than when the birds are crossed.

(9) Pullets producing most cases of the disease in their offspring have remained healthy and birds in all stages have failed to transmit the disease.

(10) These findings are obviously of limited value but suggest that a typical virus is not the cause of the condition.

(11) Lymphomatosis seems to be a condition associated with individual birds.

INCIDENCE OF IRITIS IN UTAH AGRICULTURAL EXPERIMENT STATION FLOCK

In Utah it is not uncommon to note various degrees of iritis in both the female and male birds of breeding flocks and the question has arisen as to whether or not such a flock should be used for breeding purposes.

An opportunity to study the possible danger involved in the use of breeding hens affected with iritis was provided when the Utah Agricultural Experiment Station flock developed a rather high percentage of iritis cases. Although no systematic check on the existence of disease in this flock was made prior to 1929, the caretakers in charge state that there was little if any paralysis as far back as 1920. Careful observations for eye lesions were never made prior to 1934, but caretakers recall no observance of so-called "gray-eye" or blindness, For many years no new blood had been added to the Station fflock although outside stock was annually entered in the Intermountain Egg Laying Contest, which was conducted at the poultry plant, and which offered a possible means of disease introduction. In the spring of 1932, 500 baby chicks were purchased from a breeder in the Northwest. They were held for about three weeks in a battery brooder and were then mixed and reared with chicks from the Station flock. No record is available on the incidence of iritis, if there was any, the following season (1933), but a careful individual examination of year-old hens and examination of a lesser number of two-year-old hens revealed in the following season (1934) that a considerable number were affected with iritis. Each bird in the 22 pens studied was handled and the eyes examined at monthly intervals for the months of March, April, May, and June. A total of approximately 1,100 birds were involved in the study. The incidence of disease was considerably higher in some pens than in other. Eleven percent of these birds showed definite eye lesions. In this study of affected birds, 60.5 percent showed involvement in both eyes while 39.5 percent showed only one eye affected. In a period of 60 days 14 percent of the birds affected in one eye developed the condition in both eyes. The death loss among 122 iritis-affected birds over a seven-month period was

28 (23 percent). A more recent examination in this flock of 526 year-old birds revealed that 6.8 percent showed some degree of eye involvement in April, 1937.

In this study iritis was found to be characterized by depigmentation of the iris in various degrees. The iris of the S.C. White Leghorn is normally reddish-bay color, although occasionally a bird is observed to have muddy-yellow colored iris. The depigmentation of the iris usually begins at the pupil and may extend over the entire iris leaving it a grayish color although sometimes the depigmentation begins over the entire iris at once with no special zone of discoloration adjacent to the pupil. The pupil opening may be contracted in irregular outlines. More rarely is the pupil over dilated. Sometimes the eye takes on a bulging appearance which is apparently caused by increased fluids within the anterior eye chamber.

There was observed a characteristic of some eyes which for want of a better term was called "indistinct" because of the rather blurred appearance of the eye. In normal birds the colored iris terminates at the pupil by a black colored rim which leaves a distinct line of demarcation between the dark pupil and the brown colored iris. In some birds the line of demarcation is blurred and not distinct. The black rim surrounding the pupil seems to fade and in some instances there is an irregular fading along the pupil margin of the colored iris itself so that instead of displaying a definite zone of iris decolorization, the picture is one of "indistinctness." The eye does not have a "sharp" appearance. The incidence of indistinctness on four examinations of this flock averaged 12.5 percent while the incidence of definite depigmentation for the same period was 11.0 percent. It is conceivable that this "indistinctness" is an early stage of definite iris involvement although in this study only six "indistinct" birds have later shown definite iris depigmentation. A large majority show no further progression, in fact on reëxamination one or two months later, the "indistinctness" may have disappeared entirely. It is not known therefore, what relationship, if any, "indistinctness" may bear to lymphomatosis.

Effect of iritis on progeny

Fifty-three hens showing definite iritis lesions and three hens showing "indistinct" eyes were mated with apparently normal males. The eggs produced were placed in an incubator which at the time of setting also contained hatching eggs for the routine college flock breeding program. Five days before the eggs from the iritis affected hens were hatched, they were removed from the incubator, submerged in iodine suspensoid solution (Merk) previously raised to the incubator temperature, and then transferred to thoroughly cleaned and disinfected small-unit incubators where they were hatched. There were 112 chicks hatched from eggs laid by 41 hens. The chicks were pedigree-banded and immediately moved (April 24, 1934) to a battery brooder in the veterinary building, where there would be no contact with other fowls or with caretakers of other fowls. They were later moved to a clean, coarse gravel yard not previously occupied by fowls. The poultry house had been used previously, but had been moved to the new location, remodeled, thoroughly cleaned, and disinfected. During the next few weeks there was death loss from accidents and other extraneous cause so that by July there were 65 chicks alive, the progeny from 27 iritis-affected hens and three "indistinct" hens.

The offspring included 37 cockerels, most of which were sold at the age of three months. Fifteen of these birds exhibited the "indistinct" characteristic at some examination, but not consistently so over the period studied. Twenty-seven pullets were raised

to maturity. Six examinations were made during the 13-month period over which they were observed. One pullet developed a lymphoid subcutaneous tumor on the head and showed a contracted non-responsive pupil. It died at the age of six months. One pullet showed "indistinct" on all examinations made, and three others showed this characteristic, but not consistently. In this entire study it was the practice to make the notation following examination, with no knowledge as to what had been found on previous examinations. Differences noted on various examinations may not be a true difference because in many cases very slight "indistinct" involvement might leave one in doubt as to a decision. The character of light available and other factors no doubt also contribute to irregular findings on a series of examinations. There was only the one pullet which developed a truly characteristic eye lesion. There were no cases of paralysis. None of the birds were autopsied prior to sale which was after 13 months' observation.

Progeny—F_2

In order to study the effect on the second generation the above pullets were pen mated with four males (brothers). Two of these males had "indistinct" eyes, while two had normal eyes. One-hundred fifty-seven chicks were hatched. The procedure was similar to that followed the previous years except that the eggs were not immersed in iodine suspensoid. There was again considerable loss from extraneous causes. A number of the males succumbed at 86 days of age as a result of caponizing. Seventy birds, including cockerels and capons, were observed until 10 to 13 months of age. One bird developed an "indistinct" eye at seven months of age and another developed leg paralysis at the age of 10 months. No macroscopic lesions of lymphomatosis were present in this paralyzed bird on autopsy. No cases of iritis developed in this group of birds. A post-mortem examination was conducted on all of the 70 birds at the time the experiment was concluded (after 10 to 12 months' observation) and one bird was found to have a large friable liver with whitish foci on its surface and throughout its substance. The spleen was also enlarged and friable. The lesions indicated leucosis.

DISCUSSION

In as much as the trouble with iritis in the Experiment Station flock became particularly noticeable two years after outside stock had been introduced, it suggests that the increased incidence, if there was an increased incidence, was in some way connected with the introduction of such stock. In the same year (1934) that iritis was noted to be so prevalent it was first noted that there was a considerable number of deaths exhibiting on autopsy a large friable lymphomatosis liver. It is interesting to note that during the interval June, 1933, to June, 1936, there were only 13 cases of paralysis recorded in the entire Experiment Station flock and yet during this same period cases of iritis and hepatic lymphomatosis have been numerous. Over this period records show that in birds four months or older, 28.4 percent of all death loss was due to hepatic and spleenic lymphomatosis. This experience indicates that the infective agent may, in certain circumstances, produce pathological changes in at least a portion of the hematopoietic organs to the exclusion of direct nerve tissue involvement. It again brings up the question of the tissue predilection of the infective principle.

The status of birds showing the "indistinct" and dull eye is one which requires further study, particularly from the standpoint of histo-pathology. Since several re-examinations of such birds do not always result in uniform findings it is quite likely that in many instances the condition is more

or less transitory and that it has no connection with lymphomatosis of the iris. The loss from lymphomatosis in the general Station flock during the 1934 season was 3.2 percent as compared with the loss of 1.5 percent (one bird) in the progeny from 27 iritis hens.

Since the incidence of this disease was less in strictly iritis progeny than in the general flock progeny, the results do not suggest transmission of the disease through the egg, although as mentioned by Patterson, if the disease is egg transmitted, iritis affected hens with no generalized internal lesions are not as likely to transmit the disease as are hens which have lesions of reproductive organs or lesions of the liver. Birds with lesions in these internal organs however are much less likely to produce eggs. Since there was one case of lymphomatosis in the first group of progeny and two cases in the second generation, and since the birds had no known direct exposure, the proposition may be advanced that the disease was introduced through the egg. Such reasoning is not infallible however since the possibility of disease introduction by vermin, sparrows, or other carriers cannot be ignored. The college flock, which is cited as a control, was reared under somewhat more favorable range conditions than the iritis-progeny which were confined to a small run 40 feet square.

The results in the main do not agree with those obtained by the early work of Doyle (1929), and in the later report of Patterson (1932). Since these workers used breeders from flocks badly infected with various types of lymphomatosis it is possible that they were using hens which, because of the tissues involved, were more likely to transmit the disease through the egg than are hens selected only on the basis of eye lesions; a direct comparison of results is therefore not necessarily tenable. Considering that both the control group and the iritis group in this study were known to have the disease present it is somewhat difficult to measure the results. The study does show however, that iritis-affected hens are not necessarily progenerators of lymphomatosis in their progeny; in fact, there is some evidence that their progeny may show a lower incidence of lymphomatosis.

REFERENCES

Biely, J., and Palmer, V. E., 1932. Inheritance of resistance to fowl paralysis (Neuro-lymphomatosis gallinarum). Science 78:2011, 42.

Dalling, Major T., and Warrack, G. H., 1936. Observations on fowl paralysis (Lymphomatosis). Veterinary Jour. 92:310-321.

Doyle, L. P., 1926. Neuritis in chickens. Am. Vet. Med. Assoc. 68:622.

Doyle, L. P., 1929. Neuritis or paralysis of fowls. Poul. Sci. 8:159-160.

Gildow, E. M., Williams, J. K. and Lampman, C. E., 1936. The transmission of fowl paralysis (Lymphomatosis). Poul. Sci. 15:244-248.

Johnson, E. P., 1932. A study of lymphomatosis of fowls. Virginia Agr. Exp. Sta. Tech. Bull. 44.

Kaupp, B. F., 1921. Paralysis of the domestic fowl. J. Am. Assoc. of Instructors and Invest. in Poultry Husbandry, 7:25. (As recorded by Pappenheimer.)

Pappenheimer, A. M.; Dunn, L. C., and Cone, V. A., 1926. Study of fowl paralysis. Storrs, Conn. Agr. Exp. Sta. Bul. 143:185-290.

Patterson, F. D.; Wilcke, H. L.; Murray, C., and Henderson, E. W., 1932. So-called range paralysis of the chicken. Jour. Amer. Vet. Med. Assn. No. 34:747-767.

The Size and Shape of Turkey Eggs*

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A REVIEW of the available literature does not reveal any comprehensive data on the size or dimensions of turkey eggs. During the spring of 1937 all the eggs laid from February 16 to May 7 by 20 Bronze pullets which were used as breeding stock by the Missouri Agricultural Experiment Station were weighed to the nearest gram and the diameter and length of each egg determined to the nearest millimeter. While the period covered is short and the stock was limited to the Bronze variety in their first season's production, the data were taken during the season when most turkey eggs are produced and from stock generally used for breeding purposes. While the birds used in this investigation were not trap-nested, the eggs laid during weekly periods may be compared. It is hoped that the data may be useful to those desiring such information. However, the factors affecting the size and shape of turkey eggs should be more carefully investigated.

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The relationship of the weight of turkey eggs to their diameters was shown by a correlation analysis of 200 eggs selected at random from the 768 eggs which gave r = .895, and the relationship of weight to length by a similar analysis which gave r = .753. Apparently there was a greater association between the weight of the turkey egg and the diameter of the egg than between the weight of the turkey egg and its length.

Table 2.—*Variability in size and shape of bronze pullet turkey eggs, 1937*

Date Laid	Coefficients of Variability		
	Weight	Diameter	Length
Feb. 16–March 11	8.19	3.01	3.54
March 12–18	8.06	2.84	3.95
March 19–25	9.38	3.29	4.24
March 26–April 2	7.84	2.74	3.47
April 3–9	5.61	2.42	2.37
April 10–16	5.42	2.40	2.63
April 17–23	7.44	2.94	3.08
April 24–30	8.72	3.10	3.61
May 1–7	9.60	3.64	3.52

The mean weight of 768 turkey eggs as shown in Table 1 was 84.5 grams with a standard deviation of 6.82 grams. Sixty-

Table 1.—*Turkey egg Measurements, Bronze pullets, 1937*

Date Laid	No. eggs	Weight (grams)		Width (mm.)		Length (mm.)	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
Feb. 16–Mar. 11	197	83.6	6.85	48.2	1.45	66.7	2.36
March 12–18	103	83.4	6.72	48.2	1.37	66.3	2.62
March 19–25	61	81.9	7.68	48.1	1.58	66.1	2.80
March 26–Apr. 2	69	84.4	6.62	48.5	1.33	66.8	2.32
April 3–9	69	85.8	4.82	48.8	1.18	67.1	1.59
April 10–16	75	85.7	4.64	48.8	1.17	66.8	1.76
April 17–23	77	85.1	6.33	49.0	1.44	66.3	2.04
April 24–30	71	86.4	7.53	49.3	1.53	66.7	2.41
May 1–7	46	87.7	8.42	49.4	1.79	66.7	2.35
Total	768	84.5	6.82	48.6	1.49	66.6	2.32

eight percent of these eggs were therefore included in the range from 77.68 grams to 91.32 grams. The mean diameter was 48.6 millimeters with a standard deviation of 1.49 millimeters. Therefore, 68 percent of these eggs had diameters ranging from 47.11 mm. to 50.09 mm. The mean length was 66.6 millimeters with a standard deviation of 2.32 millimeters and therefore 68 percent of these eggs varied in length from 64.28 millimeters to 68.92 millimeters. If the eggs measured in this investigation are representative of the whole population, we may expect more than 99 percent of the eggs to range in weight from 64 grams to 105 grams, to vary in diameter from 44 millimeters to 53 millimeters and to vary in length from 60 millimeters to 74 millimeters.

The coefficients of variability for the weight, diameter and length of turkey eggs as presented in Table 2 showed that diameter and length varied less than weight. The variability of all of these factors was greater from February 16 to April 2 and from April 17 to May 7 than from April 3 to April 16.

The Transmission of Xanthophylls in Feeds to the Yolk of the Hen's Egg*

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(Received for publication May 24, 1937)

IT IS recognized by investigators in egg quality that yolk color in the hen's egg is determined largely by the amount of pigment in the feed consumed by the hen. Furthermore, in a review of the literature on the coloring matter in egg yolk, Mattikow (1932) points out that the pigment in egg yolk is derived largely from the xanthophylls of plants and is closely related to these compounds. This information makes it possible for the producer to control the egg yolk color by his feeding practice.

There are, however, other factors which must be considered in attempting to control egg yolk color. Among these are the effect of the relative quantity of xanthophylls consumed on the increased color in the egg yolk and the time factors involved in the gain and loss of color of the egg yolk. It is the purpose of this report to attempt to clarify these points.

EXPERIMENT 1

Twelve lots of six White Leghorn pullets were confined to individual battery compartments. An all-mash feed mixture supplemented with granite grit and water was provided for these birds at all times. The ration consisted of 15.0 pounds ground barley, 18.0 pounds red millrun, 10.0 pounds standard wheat middlings, 15.0 pounds ground white wheat, 2.5 pounds dried skimmilk, 3.5 pounds meat scrap, 3.5 pounds fish meal, 1.0 pound steamed bone meal, 0.5 pound salt, and 1.0 pound ground oyster shell. A sufficient quantity of ground heavy oats was added to this mixture to bring the weight to 100.0 pounds and in addition, 0.8 pounds of concentrated cod liver oil was added to each 100.0 pounds of the mash. A substitution of various levels of experimental feeds was made for corresponding quantities of ground oats. The following outline of variables in the ration indicates the plan of the experiment:

Lot	*Variable*
1	Negative control
2	4% dehydrated alfalfa leaf meal
3	8% dehydrated alfalfa leaf meal
4	12% dehydrated alfalfa leaf meal
5	10% Argentine yellow corn (flint)
6	20% Argentine yellow corn (flint)
7	30% Argentine yellow corn (flint)
8	10% domestic yellow corn
9	20% domestic yellow corn
10	30% domestic yellow corn
11	4% dehydrated alfalfa leaf meal plus 10% Argentine yellow corn
12	8% dehydrated alfalfa leaf meal plus 20% Argentine yellow corn

The eggs produced by the birds receiving these rations were collected and broken out daily and the yolk colors matched with the color standards developed by Heiman and Carver (1935). All eggs laid for a three-day period prior to the feeding of the experimental rations were examined to determine the degree of pigmentation in the egg yolks at the start.

The effect of feeding various levels of domestic and Argentine yellow corn and dehydrated alfalfa leaf meal are shown in Figures 1 and 2. These curves are based on

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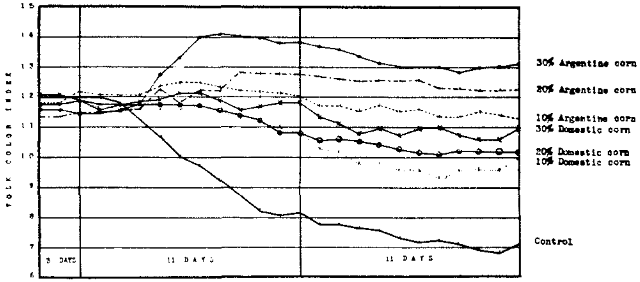

FIG. 1. The effect of domestic and Argentine corn on yolk color.

three-day running averages of the observed values. The yolk color of the eggs produced during the three-day preliminary period was quite uniform. Where yolk color was affected to any marked extent by the rations fed, the color change was noted in from two to four days. The rate of increase in the yolk color was very rapid, reaching a peak in three or four days, and then gradually falling to a fairly stationary value. Where yolk color decreased, the loss of pigment was relatively slow.

A study of Figure 1 reveals that the color imparted to the egg yolks by the domestic yellow dent corn was much less than that by the Argentine yellow flint corn. However, with both supplements the first 10 percent of the supplement fed had the greatest significance in the color change of the yolks as measured with the Color Roter. The same observation may be made for the color curves presented in Figure 2.

The xanthophylls deposited in the egg yolks from the feeding of alfalfa were considerably greater than those transmitted by either of the corn supplements and the amount of pigment transmitted by 12 percent dehydrated alfalfa corresponded close-

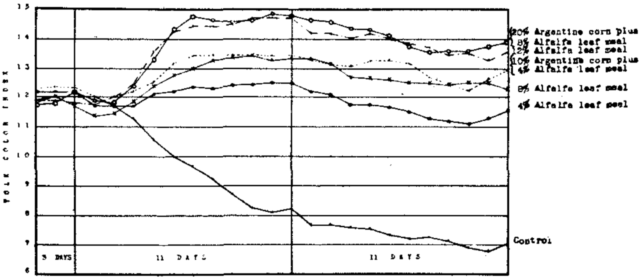

FIG. 2. The effect on yolk color of alfalfa alone and with Argentine corn.

ly to that transmitted by 20 percent Argentine yellow corn supplemented with 8 percent of alfalfa. These results indicate that as the quantity of xanthophylls transmitted

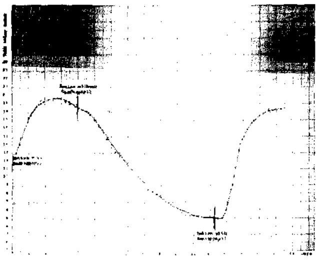

FIG. 3. A cycle of the production and loss of yolk color in relation to time.

to the egg yolk are increased, larger quantities of the xanthophyll pigments in the ration are required to produce noticeable changes in egg yolk color. It appears therefore that yolk color is not an arithmetically additive function of the xanthophylls consumed.

EXPERIMENT 2

Following this experiment another lot of 12 White Leghorn pullets was selected for a continuation of the study of the yolk color curve resulting from the addition and removal of xanthophylls in the diet. The rations used for this work are shown in Table 1.

The ration containing xanthophylls was fed for a period of 16 days to pullets which had an initial yolk color index of 13. Following this, the xanthophyll-free ration was fed for a period of 33 days, and then the ration containing xanthophylls was again fed for 18 days.

The results of this work are shown in Figure 3 and a color cycle extending from the minimum to the maximum colors observed with the rations fed has been developed. This color curve shows the rate of gain and loss of pigment from the yolk. The curve is plotted to fit as nearly as possible, points determined by a three-day running average of the observed values.

From a study of this curve the observations of the first experiment are confirmed in that the increase in yolk color is very rapid, whereas the loss of xanthophylls from the egg yolks is more gradual.

SUMMARY

1. The first increment of xanthophylls present in the ration of laying hens produced a greater change in yolk color than additional corresponding increments.

2. The yolk pigmentation observed with dehydrated alfalfa leaf meal was greater than for a corresponding quantity of corn used in this experiment.

3. Argentine yellow flint corn caused the production of a greater quantity of yolk pigment than a corresponding quantity of domestic yellow dent corn.

4. A color curve was developed showing the relationship between time and yolk color

TABLE 1.—*Rations used*

With Xanthophylls
60 pounds Argentine yellow flint corn
15 pounds dehydrated alfalfa leaf meal
15 pounds dried skimmilk
5 pounds dried yeast
2 pounds ground oyster shell
1 pound bone meal
1 pound salt
1 pound concentrated cod liver oil
Without Xanthophylls
70 pounds ground white corn
17 pounds dried skimmilk
3 pounds wood fiber
5 pounds dried yeast
2 pounds ground oyster shell
1 pound bone meal
1 pound salt
1 pound concentrated cod liver oil

when hens received diets free from or containing xanthophylls. This curve confirms the observation that the increase in yolk color is very rapid, whereas the loss of xanthophylls from the egg yolk is more gradual.

REFERENCES

Heiman, V. and Carver, J. S., 1935. The yolk color index U. S. Egg and Poultry Magazine, 41:40-41.

Mattikow, M., 1932. A critical review of the literature on the coloring matter in egg yolk. Poul. Sci. 11:83-93.

News and Notes

The Acting Editor of POULTRY SCIENCE regrets that he failed to announce in the September issue of the Journal that Michael Lerner of the Poultry Division of the University of California was awarded the Poultry Science Research Prize for his paper entitled, "Relative Growth and Hereditary Size Limitation in the Domestic Fowl" and that honorable mention was given to S. S. Munro of the Dominion Experimental Farms for his paper entitled, "The Inheritance of Egg Production in the Domestic Fowl."

Professor J. Holmes Martin of the University of Kentucky has been given charge recently of the work in genetics at that Institution. In addition to these new duties Professor Martin will continue to have supervision of the work in Poultry Husbandry.

Dr. A. T. Ringrose has resigned his position with the National Oil Products Company to accept one with the University of Kentucky as Instructor of Poultry Husbandry. Dr. Ringrose will give about three-fourths of his time to teaching and the remainder to research work.

Professor J. E. Humphrey of the University of Kentucky who for the past year has been on leave of absence with the National Poultry Improvement Plan returned to his duties as Extension Poultry Husbandryman November 1.

Mr. Wilbor O. Wilson has recently joined the Poultry Department of South Dakota State College. Mr. Wilson came from the Larro Farms, Detroit, Michigan. He received his B.S. degree from Oklahoma A. & M. College and his M.S. degree from Kansas State College.

Dr. Theodore C. Byerly joined the staff of the Poultry Department of the University of Maryland, October 1. Dr. Byerly will have immediate charge of the research program of the Department. He was formerly connected with the National Agricultural Research Center at Beltsville, Maryland.

Dr. Malcolm Lyons has resigned his position with the Poultry Department of the University of Kentucky to accept one with the University of Arkansas. Dr. Lyons entered upon his new duties July 1.

Mr. Marshall E. Coe, former member of the teaching staff at Connecticut State College and at Mississippi, and commercial poultryman since 1930, has been appointed assistant poultry husbandman of the Virginia agricultural extension division, effective September 16.

The Influence of Fat-Soluble Vitamins on Egg Production and Egg Shell Composition

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(Received for publication May 25, 1937)

IN ORDER to produce eggs profitably, the commercial poultryman must secure a high production of eggs with shells of a texture that will successfully withstand shipping and storage conditions. To accomplish this the poultryman must constantly supply his birds with rations of high nutritive efficiency. Such rations must contain a sufficient component of vitamins to insure satisfactory utilization of the essential constituents of the ration.

Lately some attention has been given to determining the value of the fat-soluble vitamins for supplementing the ration of mature birds but in many of these studies the rations used were compounded along experimental rather than practical feeding lines. Accordingly it seemed desirable to determine the influence of fat-soluble vitamins for improving egg production and egg shell composition of hens subsisting upon a ration similar in composition to high quality commercial laying rations.

EXPERIMENTAL

This study was conducted with well matured, Rhode Island Red pullets from disease-free stock. They were grown from day-old chicks under controlled laboratory conditions. At the beginning of this study the pullets were 20 weeks old, nicely feathered, weighed four and three-quarters pounds, and were just commencing to lay. The pullets were divided into three groups of 24 birds which were identical as regards weight, appearance, pigmentation, and previous nutrition.

The battery room had an eastern and southern exposure but southern exposure did not affect the portion of the room used for this study. It will be noted (Fig. 1) that the laying batteries were arranged parallel to one another and perpendicular to the eastern exposure. Thus, only a few birds in the end compartments, which were equally distributed through the different groups, received direct sunlight, after it had passed through window glass, for only a short time during early summer mornings. Hence the experimental birds were maintained under far more severe conditions than birds in commercial poultry plants. The battery room was provided with steam heat but this was used in only one or two instances of severe weather. In general the temperature of the battery room was largely regulated by the outside temperature. During the winter months the temperature of the battery room was never permitted to drop as low as 32 degrees F. No attempt was made to curtail the duration of daylight during the summer months. In the winter a sufficient period of artificial lighting was used to lengthen the winter days to 13 hours. Care

was observed in providing identical lighting for each group of birds.

The pullets were housed in all-metal laying batteries (Fig. 1). Each pullet lived in a separate compartment. The compartments were 12 inches by 18 inches by 16 inches high and thus provided two cubic feet of space. The floors of the compartment sloped towards the front and were made of wires spaced one inch apart. Hence the pullets were at all times out of contact with the litter and their droppings. Each bird was provided with an individual feed hopper but shared a drinking fountain with the bird in the next compartment.

The basal ration was compounded of readily available materials such as are commonly used in high quality rations. It contained the following ingredients:

Basal Ration

	Percent
Yellow corn meal	40.2
Wheat bran	16.2
Wheat flour middlings	14.6
Ground oats	11.6
Dried Skimmilk	4.2
Alfalfa leaf meal	4.2
Meat scraps (50% protein)	3.0
Fish meal (58% protein)	3.0
Oyster shell meal	2.0
Dicalcium phosphate	0.5
Salt	0.5

A sardine body oil produced under modern manufacturing conditions, from fish caught off the California coast, furnished supplementary vitamin A and vitamin D. The vitamin potency of this oil was approximately 500 Vitamin A units and 100 Vitamin D units (U.S.P. XI) per gram. At this point it should be noted that the alfalfa leaf meal contributed materially to the vitamin value of the basal ration and Capper, McKibbin, and Prentice (1931) have established the ability of the hen to convert carotene into vitamin A. The alfalfa leaf meal supplied 70,000 or more units of carotene per pound, as determined by the Guilbert (1934) method. Hence, the basal ration, by carotene analysis, and referred to U.S.P. XI units, contained over 4,000 units per pound.

The basal ration was divided into three lots designated lot 1, 2, and 3. One percent, one and a quarter percent, and two percent respectively of sardine oil was carefully incorporated in the three lots of basal ration. All the birds consumed the all-mash ration ad libitum. During the experimental period, the birds of groups 1, 2, and 3 consumed an average of 2.21 lbs., 2.14 lbs., and 1.95 lbs. of oyster shell and 2.11 lbs., 2.04 lbs., and 1.97 lbs. of granite grit respectively.

FIG. 1. Battery room showing arrangement of birds and provision for feed and water.

The experimental period was of 12 months' duration. It has been commonly observed that pullets produce more eggs during the early than during the late laying period. Furthermore well-nourished, fully matured pullets may for a time draw upon body reserves to compensate for small deficiencies in their ration. Hence in order to determine the significance of differences in the nutritive value of rations the experimental period should be of adequate length. Accordingly in this study observations were made for a 12 months' laying period.

The pullets were weighed at four-week intervals. The individual weights of the 72 birds at 14 periods for weighing are too voluminous to be reproduced here. Accordingly the weights for each group of birds

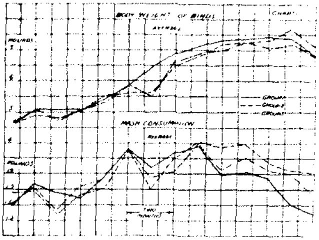

CHART 1. Growth and mash consumption curves for the three groups of pullets.

were averaged and these values have been plotted as growth curves (chart 1). As was to be expected, the body weight of the pullets increased for practically the entire experimental period. The average weight was very similar for the three groups and it increased very consistently for the whole year.

The mash consumption was determined at weekly intervals. The average consumption for each of the three groups of birds was computed weekly. These data are reported as curves in chart 1. The average mash consumption for each group showed considerable fluctuation. The total amount of mash consumed per bird during the experiment was 99.7 lbs. for group 1, 103.4 lbs. for group 2, and 100.0 lbs. for group 3.

The mortality for the 52-week experimental period was three birds for each of the three groups. Three other birds of group 1 were removed during the experiment since they were out of condition. In this study the mortality was due to a variety of conditions, that is, in one bird the oviduct was ruptured, in one the ureter was blocked, one bird had paralysis and all internal organs were atrophied, another had a tumor in the cloaca, in one the oviduct was obstructed, and another had an abnormal liver and ulcers in the proventriculus.

The egg production for the three experimental groups of birds, during the period July to May inclusive, was computed on an average, percentage basis. These computed monthly data have been plotted as curves which appear in chart 2. It will be noted that the egg production for the period July to December was relatively high and that thereafter there was a consistent decrease. The average egg production for the entire experimental period was least for group 1 and largest for group 3. In other words, the number of eggs produced increased with the increase of sardine oil contained in the experimental rations. It is also interesting to note that group 3, which received 2 percent of sardine oil in the basal ration, maintained a more even egg production than either of the other two groups. These results with sardine oil are in agreement with those reported for cod liver oil. Edson (1932) conducted a three-year experiment during which 1, 2, and 3 percent of cod liver oil was fed as a supplement to the mash. He

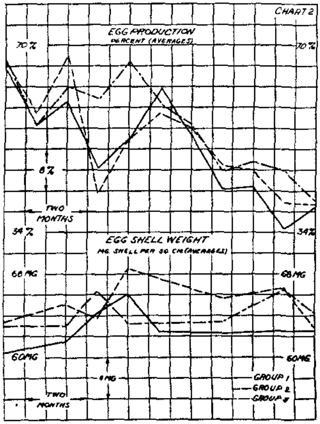

CHART 2. Curves for egg production and egg shell weight determined at intervals during the year's experiment.

found that cod liver oil increased egg production and that 2 percent of oil was the most economical amount to add to the ration. Sherwood and Fraps (1934) were able to increase egg production 15 percent by increasing the vitamin A (alfalfa) content of the ration. Murphy, Hunter, and Knandel (1934) also found that, within certain limits, increasing the fortified cod liver oil supplement to the ration increased egg production.

Eggs were collected, from each hen in production, at seven intervals during the experimental period, namely, July, September, October, November, February, April, and May. These eggs were used for determining the amount of shell per unit area. In a study of the relationship between the breaking strength and the percentage of egg shell Morgan (1932) found that "A definite positive correlation exists between the percent shell of an egg and its breaking strength." Accordingly in this study the thickness or strength of eggshells was judged by the weight of shell per unit surface area. The surface area of each egg was computed by the formula, Surface area = 5.07 × (weight of egg)$^{2/3}$, developed by Murray (1925). It is quite apparent from the formula that the weight of the egg is a highly important factor in obtaining an accurate value for the surface area. Accordingly, data were assembled concerning possible change in weight of eggs during early storage. For this purpose 12 representative eggs, weighing from 47.87 grams to 61.27 grams, were stored in an ordinary one dozen egg box at laboratory temperature. On the day this study was initiated all eggs were removed from the laying batteries at 8:00 A.M. The 12 eggs used in this study were collected before noon. Hence they were strictly fresh eggs. Since data concerning the first days of storage were particularly important the test was started on Monday and continued for seven days. The daily weights of all eggs are reported as curves in chart 3. Inspection of the curves will reveal they lost weight consistently. The greatest loss during the first seven days' storage was 2.27 percent for the egg initially weighing 53.56 grams and the least loss was 1.24 percent for the egg weighing 48.54 grams. The average loss in weight of the twelve eggs during seven days

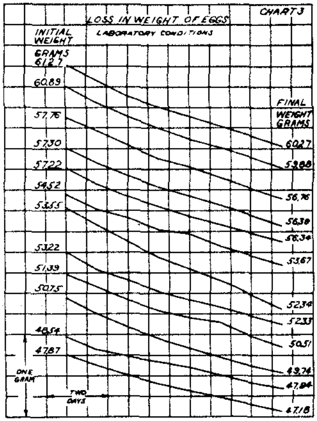

CHART 3. Curves for loss of weight of eggs during early storage under laboratory conditions.

storage was 1.67 percent. As was expected the percentage loss in weight was more for the larger than for the smaller eggs. It was quite evident from these data that the eggs used in the shell thickness study should be weighed under uniform conditions. Accordingly all eggs included in the shell study were weighed within three hours after they were laid.

The weight of the egg shell was obtained by boiling the eggs 10 minutes, allowing them to cool, carefully removing the shell from the underlying membrane, heating the shells 16 hours at 100°C., cooling in a desiccator and weighing. The weight of shell per square centimeter of shell was determined

TABLE 1.—*Egg production and shell weights for individual birds—group 1. Weight of shell per square centimeter of shell*

Pullet No.	Egg record	July eggs	Sept. eggs	Oct. eggs	Nov. eggs	Feb. eggs	April eggs	May eggs
	1 year	mgs.	mgs.	mgs.	mgs.	mgs.	mgs.	mgs.
25	205	71.10	68.11	68.30	74.25	71.31	70.92	75.97
26	182	60.03	53.70	58.62	69.34	65.70	63.60	54.89
27	195	54.75	63.21	—	69.19	63.60	55.07	52.65
28	55	59.62	53.73	—	—	—	—	—
29	226	56.80	61.04	59.38	58.06	52.14	54.24	54.76
30	229	44.17	47.59	50.76	42.77	43.94	—	—
31	238	62.01	65.73	—	71.74	68.70	71.56	61.79
32	150	65.17	52.58	59.61	—	45.60	57.24	57.59
33	163	61.37	69.75	70.81	72.45	70.62	61.22	53.08
34	201	64.28	60.58	61.07	64.38	56.72	—	59.14
35	184	64.67	66.21	51.72	64.89	57.69	—	—
36	85	45.20	58.40	—	—	—	54.68	61.22
37	139	70.70	71.07	77.88	—	73.61	51.94	73.34
38	144	47.99	59.05	59.95	60.03	53.00	58.54	55.08
39	236	62.59	58.34	63.34	60.07	56.40	—	—
40	163	53.97	67.30	68.01	69.48	68.49	58.54	—
41	135	62.55	62.79	66.44	65.82	—	—	—
42	112	60.33	—	—	65.90	—	—	—
43	15	—	—	—	—	—	—	—
44	231	67.67	65.06	71.39	71.85	71.63	62.00	63.00
45	294	55.08	51.70	—	60.82	57.02	56.98	50.27
46	243	62.94	70.73	67.85	69.45	70.62	70.70	67.45
47	40	70.95	—	—	72.21	—	—	—
48	179	64.20	60.69	73.16	68.69	61.88	70.42	61.71

TABLE 2.—*Egg production and shell weights for individual birds—group 2. Weight of shell per square centimeter of shell*

Pullet no.	Egg record	July eggs	Sept. eggs	Oct. eggs	Nov. eggs	Feb. eggs	April eggs	May eggs
	1 year	mgs.	mgs.	mgs.	mgs.	mgs.	mgs.	mgs.
49	85	58.50	42.91	—	—	—	—	—
50	150	51.48	61.97	—	64.64	63.84	63.54	66.77
51	111	65.94	72.01	66.53	63.68	—	67.19	60.29
52	197	67.76	66.69	—	67.73	—	—	62.24
53	169	66.73	71.90	—	65.89	69.00	70.25	58.63
54	236	71.47	66.22	66.95	78.44	76.60	77.92	66.23
55	147	66.14	65.06	—	63.49	56.51	—	59.61
56	235	60.66	68.39	53.53	73.96	71.68	62.99	69.36
57	215	62.71	60.42	—	74.81	64.62	75.90	59.19
58	260	68.07	64.94	69.51	65.73	67.58	61.94	60.32
59	62	65.85	68.19	—	—	—	—	—
60	136	63.91	71.02	—	66.42	68.29	[illegible]	64.01
61	161	61.55	66.83	69.06	75.01	62.57	[illegible]	60.94
62	223	63.86	66.07	64.81	—	—	—	51.26
63	199	69.54	70.19	67.86	70.70	69.45	67.44	64.66
64	188	56.62	69.46	67.34	72.80	67.32	63.04	66.86
65	251	76.09	67.97	61.13	73.23	69.58	69.42	64.80
66	258	67.71	66.81	73.20	64.56	69.89	69.33	61.76
67	231	60.12	60.43	58.12	69.00	68.08	71.65	69.98
68	273	64.49	64.58	50.49	64.90	61.10	52.61	60.07
69	207	65.69	71.13	70.19	74.52	62.00	72.09	61.78
70	161	41.26	60.13	58.93	67.29	—	62.73	61.30
71	72	69.33	69.96	—	—	—	—	—
72	149	55.63	49.13	59.09	57.19	54.49	—	—

from the total weight of the dried shell and the previously determined surface area of the egg. The average values for weight of shell per square centimeter of surface for each group of birds at the seven intervals during the experiment are plotted as curves in chart 2.

The weight of egg shell per square centimeter of shell was least for group 1 and largest for group 2. From these data it might be assumed that the shells of eggs from group 2 were heavier than those of group 1 on account of the additional sardine oil consumed. However, the further increase of sardine oil consumed by group 3 did not enable group 3 to produce eggs with as heavy shells as those of group 2.

While the curves referring to egg shell thickness, chart 2, supply a convenient summary of the analytical data, they do not show the variation in egg shell thickness for different birds at the same time interval nor do they reveal the variation in egg shell thickness for the same bird at the different time intervals during the experimental period. Accordingly these detailed data are reported in Tables 1, 2, and 3. A record of the egg production of each bird is also included in order to facilitate a correlation of the egg production and egg shell weight.

TABLE 3.—*Egg production and shell weights for individual birds—group 3. Weight of shell per square centimeter of shell*

Pullet no	Egg record	July eggs	Sept. eggs	Oct. eggs	Nov. eggs	Feb. eggs	April eggs	May eggs
	1 year	mgs.	mgs.	mgs.	mgs.	mgs.	mgs.	mgs.
121	224	73.85	70.79	67.91	69.89	62.88	—	—
122	165	62.74	68.40	74.08	64.57	65.89	65.71	61.44
123	189	65.28	—	58.77	49.64	46.98	—	—
124	171	—	64.89	62.78	73.46	65.78	68.55	—
125	195	54.00	50.43	60.20	61.91	57.98	—	59.28
126	207	66.11	58.53	76.64	68.65	69.54	56.78	63.10
127	243	61.09	68.35	66.19	61.88	63.55	62.55	80.07
128	264	62.69	62.96	63.98	67.42	65.24	61.17	65.10
129	180	63.74	68.11	66.24	65.41	57.48	68.94	64.16
130	203	68.06	69.30	61.50	70.76	69.66	68.27	65.83
131	198	60.93	62.97	62.26	62.42	66.26	63.89	—
132	172	47.61	45.75	54.73	61.17	—	—	42.78
133	236	59.41	61.40	63.75	61.23	61.60	61.13	64.23
134	254	64.17	62.23	72.77	67.45	59.89	64.11	60.01
135	79	70.34	66.22	—	—	—	—	—
136	151	66.02	—	66.16	63.56	65.70	65.14	58.79
137	188	—	70.39	73.08	—	80.04	76.34	69.60
138	198	62.19	72.07	58.74	46.68	72.43	67.30	—
139	46	—	—	—	—	—	—	—
140	167	68.54	64.83	84.86*	—	—	80.32	74.26
141	232	56.12	58.51	66.11	59.28	55.81	—	—
142	181	72.11	43.89	60.10	62.14	48.10	—	—
143	225	53.63	57.04	69.11	55.98	67.98	66.64	65.89
144	122	62.95	74.68	—	72.06	—	—	—

* Double Shell.

The egg production of group 1 was 4,211 eggs, which is 49.2 percent production or 180.4 eggs per bird. Group 2 produced 4,500 eggs, which is 51.5 percent production, or 187.8 eggs per bird. Group 3 produced 4,571 eggs, which is 53.9 percent production, or 197.2 eggs per bird. These values for percent egg production were computed on a hen-day basis.

It also will be noted (Tables 1, 2, and 3) that the weight of egg shell per square centimeter of shell varied materially for the

different birds of each group, for each of the seven periods of observation. At the first test the weight of shell varied from 44.17 mg. to 71.10 mg. for group 1, from 41.26 mg. to 76.09 mg. for group 2, and from 47.61 mg. to 73.85 mg. for group 3. At the end of the year the variation was from 50.27 mg. to 75.97 mg. for group 1, from 51.26 mg. to 69.98 mg. for group 2, and from 42.72 mg. to 80.07 mg. for group 3.

It is interesting to note that some birds such as Nos. 25, 37, 54, and 137, throughout the year, produced shells that were definitely heavier than those of other birds. Similarly some birds such as Nos. 29, 30, 45, 72, and 132 produced relatively thin shells. These observations seem to suggest the possibility that selective breeding might eventually produce a strain of birds with the capacity for producing eggs with thick shells. Inspection of the data will reveal that some birds such as Nos. 31, 50, 56, 67, and 143 tended to produce heavier shells as the experiment progressed. Also some birds such as Nos. 33, 38, 40, 44, 61, 122, 126, 134, and 141 increased shell thickness for a time and during the last portion of the experiment produced shells of lesser weight. However the thickest egg shells were not obtained from the lowest egg producers nor were the thinnest shells laid by the birds with the highest egg production.

In spite of the variation in the weight of egg shells for the different members of the group the average weight of shells of each group increased for the first half of the experimental period and then decreased during the last half of the experiment. This was particularly true for group 1 which consumed the smallest amount of sardine oil. The weight of egg shells, except at the termination of the study, was definitely larger for group 2 than for group 1. The average weight of shells from group 3 was between that of group 1 and group 2 but during the last portion of the experiment it tended to increase rather than decrease as was the case for group 1 and group 2.

Averages for the weight of eggs, their surface area, the weight of the dry shell, and the weight of the shell per square centimeter for all three groups have been assembled in Table 4. Inspection of this table reveals that the average weight of eggs for the entire experiment was least for group 1 and greatest for group 3 which coincides with the increase of cardine oil in the ration. The surface area of the egg shells also increased in the same manner. The weight of dry shell was least for group 1 and greatest for group 2. The same was true for the amount of shell per square centimeter.

In order to determine the vitamin A stores of the birds at the beginning of the experiment five normal birds were selected for study. It subsequently developed that the data concerning the vitamin A reserves of the experimental birds at this stage would have been more conclusive if more birds had been included in this group. However, the data that were assembled are included here to indicate the probable vitamin A reserve at the start of the experiment. Baumann, Riising, and Steenbock (1934) have reported that 95 percent of the total vitamin A of rats is present in the liver. This observation was confirmed by Sherman and Boynton (1925), Rosenheim and Webster (1927), Moore (1931), and McCoord and Luce-Clausen (1934), and recently Cruickshank and Moore (1937) have shown that for intensive cod liver oil feeding 98.5 percent of the body reserve of vitamin A was located in the liver. Since it has been demonstrated that nearly all the body reserve of vitamin A is located in the liver this organ was assayed for its vitamin content. As soon as the birds were killed their livers were removed and ground with an equal weight of anhydrous sodium sulphate. A portion of the liver-sulphate mixture was extracted eight hours with anhydrous ether to determine the percentage of fat in the liver. Fat obtained from the extraction of

TABLE 4.—*Results of egg shell studies*
(Group Averages)

Collection date	Weight of egg	Surface area of shell	Wt. of Dry egg shell	Wt. of shell per sq. cm. shell
	gms.	sq. cm.	mgs.	mgs.
		Group 1		
July	46.54	65.60	3969.	60.50
Sept.	50.82	69.56	4278.	61.50
Oct.	54.44	73.23	4701.	64.19
Nov.	58.85	76.71	5066.	66.04
Feb.	60.35	78.01	4861.	62.31
Apr.	60.93	78.50	4909.	62.53
May	59.12	76.94	4811.	62.52
Average	55.86	74.08	4656.	62.85
		Group 2		
July	48.74	67.66	4288.	63.37
Sept.	51.56	70.23	4568.	65.04
Oct.	53.50	71.97	4596.	63.85
Nov.	58.77	76.64	5260.	68.63
Feb.	61.12	78.67	5185.	65.90
Apr.	62.09	79.49	5299.	66.66
May	60.32	77.97	4876.	62.53
Average	56.59	74.66	4867.	65.18
		Group 3		
July	47.97	66.94	4211.	62.91
Sept.	52.68	71.25	4486.	62.96
Oct.	54.80	73.14	4845.	66.24
Nov.	58.11	76.07	4817.	63.32
Feb.	60.30	77.96	4958.	63.60
Apr.	61.78	79.23	5273.	66.55
May	61.20	78.74	5064.	64.31
Average	56.69	74.76	4808.	64.31

the liver-sulphate mixture at laboratory temperature was assayed by the Carr-Price (1926) method for its vitamin A content. The results of these essays are summarized in Table 5.

It will be noted that the weight of the livers was variable and was not proportional to the body weights of the birds. Also it is interesting to note that the largest liver had the highest fat content. The average vitamin A reserve for the five birds was 83.40 Blue Units per gram of liver.

The vitamin A reserve of the birds at the conclusion of the experiment was determined by the method previously discussed by Holmes, Tripp, and Campbell (1936). For this purpose 18 birds were selected from group 1 and group 3 which received 1 and 2 percent respectively of sardine oil. In selecting birds for the liver assays they were so chosen as to represent a varied egg production. Also an attempt was made to obtain pairs of birds of approximately the same body weight and egg production. The

TABLE 5.—*Vitamin A reserve of birds at start of experiment*

Bird No.	Weight of bird	Weight of liver	Liver fat	L.B.U. per gm. liver
	gms.	gms.	%	
1	1722	27.45	4.10	89.82
2	2030	40.03	3.97	63.63
3	1890	43.19	4.63	18.95
4	1670	29.06	3.79	118.56
5	1830	27.62	3.83	126.05
Average	1828	33.47	4.06	83.40

data which were assembled for eight pairs of birds from groups 1 and 2 are reported in Table 6. There was no bird in group 3 to pair with bird No. 36 as the two low producers, Nos. 135 and 139, were not satisfactory for this purpose. Nevertheless it seemed desirable to collect data for bird No. 36. At autopsy it was found that bird No. 138, which was selected to pair with bird No. 27, had numerous tumors on the internal organs. Accordingly bird No. 137, which had a similar egg record, was selected to pair with bird No. 27. At autopsy two tumors were found on the liver. However these were removed and the liver was prepared for assay. These two birds, Nos. 137 and 138, gave definitely higher results, that is, 284.20 and 259.80 Blue Units respectively, than the other birds. On the other hand bird No. 127 was in much the same condition as bird No. 137 and her liver gave only 139.70 Blue Units per gram.

It will be noted that as the egg production of the birds in group 1 increased the vitamin A reserve of their livers tended to decrease, which confirms the finding of Russell and Taylor (1935) that the liver vitamin A varied inversely with egg production. This tendency was decidedly less for group 3. With one exception each bird of group 3 had a definitely larger vitamin A store than its mate of group 1. The average egg production was larger for group 3, which produced 211 eggs, than for group 1, which produced 202 eggs. The average weight of livers was 38.93 grams for group 1 as compared with 51.67 grams for group 3. The average fat content of the livers was 11.85 percent for group 1 and 12.81 percent for group 3. However the average vitamin A store for group 1 was only 81.2 Lovibond Blue Units as compared with 180.8 Blue Units for group 3. The vitamin A reserve of 22.1 Blue Units per gram of liver for bird No. 136 is obviously out of line with the results obtained for the other eight birds of the group. Hence it may be desirable from a statistical standpoint to eliminate this bird, and obviously its mate bird, No. 32. In that case the vitamin A reserve for group 1 becomes 81.4 units and for group 3, 207.4 Blue Units per gram of liver. However in either case it is very evident that the additional sardine oil consumed by group 3 caused the birds of group 3 to develop more than twice the vitamin A reserve of group 1.

The vitamin A potency of eggs produced by the birds of group 1 and group 3, during the last month of the experiment, was determined by the official United States Pharmacopoeia (1936) biological method. Eggs were collected daily and the yolks were carefully separated from the whites and pooled for each group. The thoroughly mixed yolks were mixed with an equal weight of anhydrous sodium sulphate. The mixture was extracted at room temperature with anhydrous, peroxide-free ether. The fat obtained was administered daily to rats maintained on the official vitamin A and vitamin D procedures. It was found that the eggs from the birds of group 1 during the last month of the study had a vitamin A potency of 214 U.S.P. XI vitamin A units per yolk and the eggs from the birds of group 3 had a vitamin A potency of more than 320 vitamin A units per yolk. These results are in accord with those reported by Bethke, Kennard, and Sassaman (1927), Cruickshank and Moore (1937), Ellis, Miller, Titus, and Byerly (1933), Sherwood and Fraps (1935), Thomas and Quackenbush (1933), Bearse and Miller (1937), and Koenig, Kramer, and Payne (1935), who found that the vitamin A content of eggs tended to increase as the vitamin A content of the ration of laying hens was increased beyond that required for body maintenance and normal egg production. On the other hand Russell and Taylor (1935) found that eggs contained only from 11 to 32 percent of the vitamin A present in a

TABLE 6.—*Vitamin A reserve of birds at end of experiment*

Bird no.	Group no.	Egg record	Wt. of bird	Wt. of liver	Liver Fat	L.B.U. per gm. liver	Autopsy record
		1 year	gms.	gms.	%		
36	1	85	3464	51.03	10.27	104.80	Laying—good condition, all organs normal.
32	1	150	3707	49.05	13.86	79.66	Laying—good condition, mucb fat.* Intestines somewhat constricted. All other organs normal.
136	3	151	2451	45.29	8.85	22.10	Laying—All organs healthy. Egg in oviduct. Medium amount of fat.*
26	1	182	2753	30.60	5.74	114.80	Not laying—grayish brown, gangrenous yolks on ovary. All other organs normal.
129	3	180	2782	34.67	6.11	145.50	Laying—good condition, egg in oviduct. All organs normal. Medium amount fat.*
27	1	195	3322	36.86	7.41	119.50	Laying—Egg in oviduct. All organs normal. Much fat.*
138	3	198	3350	60.34	17.67	259.80	Not laying—Tumors on ovary, duodenal loop, ceca and external tumor on anus. Other organs normal.
137	3	188	4028	61.78	10.23	284.20	Laying—much fat.* Two small black tumors on liver. Black piece of tissue free against ribs. Other organs normal.
25	1	205	3464	55.02	14.79	50.30	Laying—Excellent condition. All organs normal.
130	3	203	3877	74.31	27.06	187.90	Very fat.* All organs normal. Egg in oviduct. Liver large and buff color.
44	1	231	2919	46.99	8.66	71.00	Laying—All organs normal.
141	3	232	3265	55.66	12.13	216.00	Not laying—All organs normal.
39	1	236	3521	43.97	13.11	91.00	Not laying—Large cyst size of a grapefruit in oviduct. Well developed yolks on the ovary. All other organs normal.
133	3	236	3032	54.29	18.63	245.10	Laying—good condition. All organs normal. Liver buff color.
46	1	243	3521	53.54	20.36	50.90	Laying—good condition.
127	3	243	2327	31.46	7.32	139.70	Tumor on liver, piece black tissue free against ribs. Constricted intestines.
45	1	294	2919	55.36	12.41	48.85	Laying—good condition. Several small cysts on liver.
128	3	264	3117	47.23	6.85	180.30	Two unabsorbed eggs in abdominal cavity. All other organs normal.
Average	1	202	3288	36.93	11.85	81.2	
Average	3	211	3137	51.67	12.81	186.8	

* "Fat" in autopsy records means abdominal fat.

normal ration supplemented with 1 percent of cod liver oil, that the proportion of vitamin A transferred to the eggs decreases rapidly with dietary vitamin A increase and that there is no fixed ratio which is applicable to various feed and management conditions.

The vitamin D content of eggs produced by birds of group 1 and group 3 during the last month of the experimental period was also determined by the United States Pharmacopoeia biological method (1936). The vitamin D test showed that the eggs from the birds of group 1 contained 13 vitamin D units and those from birds of group 3 contained 22 vitamin D units, U.S.P. XI per yolk. In a similar study of cod liver oil as a source of vitamin D for laying hens DeVaney, Munsell, and Titus (1933) found "The vitamin D content of egg yolk was significantly greater when 2 percent of cod liver oil was included in the diet than when 1 percent was fed." Bethke, Kennard, and Sassaman (1927) have reported that 2 percent of cod liver oil added to the basal diet materially enhanced the vitamin D content of the eggs. Guerrant, Kohler, Hunter, and Murphy (1935) concluded from their studies of the ration of laying hens that there is a relationship between the amounts of cod liver oil fed and the antirachitic potency of the eggs produced.

It is quite evident from these results that the eggs from the birds of group 3 have a definitely higher food value than those from the birds of group 1. Presumably also the higher vitamin A and vitamin D content of the eggs from birds of group 3 would favorably influence the viability, bodily resistance, and growth of the chicks produced from such eggs.

SUMMARY

Three groups of 24 well matured, 20-week-old Rhode Island Red pullets were placed on a 52 weeks' test of the value of sardine body oil as a source of vitamin A and vitamin D for supplementing a well balanced laying mash. One percent, 1.25 percent, and 2 percent of sardine oil was added to the ration fed to group 1, group 2, and group 3 respectively.

Data were collected concerning mash consumption, increase in body weight of birds, mortality, egg production, egg shell thickness or weight, final vitamin A reserve of birds, and the vitamin A and vitamin D content of the eggs at the termination of the experimental period.

During the first 20 weeks all three groups weighed practically the same and increased in weight at about the same rate. During the remainder of the experiment, except for the last five weeks, group 1 weighed more than the other two groups.

The total mash consumption was 99.7 lbs., 103.4 lbs., and 100.0 lbs. per bird for groups 1, 2, and 3 respectively.

The mortality was three birds from each of the three groups. Most of the mortality occurred during the latter part of the experiment.

The egg production was fairly high for all three groups. The average production, for the 52 weeks, was 180.4 eggs for group 1, 187.8 eggs for group 2, and 197.2 eggs for group 3.

The egg shell weight increased for all three groups for about 16 weeks. Subsequently the shell weight decreased somewhat. For practically the entire experimental period the egg shell weight was least for group 1 and greatest for group 2.

The vitamin A reserve at the end of the experiment was judged by nine typical birds of groups 1 and 3. These were killed and their livers were assayed for their vitamin A content. Group 1 gave 81.2 Blue Units per gram of liver and group 3, 186.8 Blue Units per gram of liver.

The vitamin A content of eggs from group 1 and group 3, at the end of the year's test,

was determined by the official biological method. The vitamin A content of eggs from group 1 was 214 U.S.P. XI units per yolk and that of eggs from group 3 was 320 units per yolk. The vitamin D content was 13 units per yolk for group 1 and 22 units for group 3.

REFERENCES

Baumann, C. A., B. M. Riising, and H. Steenbock, 1934. Fat-soluble vitamins. XLII. The absorption and storage of vitamin A in the rat. J. Biol. Chem. 107:705-715.

Bearse, G. E. and M. W. Miller, 1937. The vitamin A requirements of White Leghorn pullets during the growing period. Poul. Sci. 16:34-38.

Bethke, R. M., D. C. Kennard, and H. L. Sassaman, 1927. The fat-soluble vitamin content of hen's egg yolk as affected by the ration and management of the layers. J. Biol. Chem. 72:695-706.

Capper, N. S., I. M. W. McKibbin, and J. H. Prentice, 1931. XXXIII. Carotene and vitamin A. The conversion of carotene into vitamin A by fowl. Biochem. J. 25:265-274.

Carr, F. H. and E. A. Price, 1926. LXIV. Colour reactions attributed to vitamin A. Biochem. J. 20:497-501.

Cruickshank, E. M. and T. Moore, 1937. XXI. Vitamin A and carotene, XVI. The effect of the administration of large amounts of vitamin A on the vitamin A content of the hen's egg. Biochem. J. 31:179-187.

DeVaney, G. M., H. E. Munsell, and H. W. Titus, 1933. Effect of sources of vitamin D on storage of the antirachitic factor in the egg. Poul. Sci. 12:215-222.

Edson, A. W., 1932. Cod liver oil in the winter ration of pullets: Results of a three-year study of effects on egg production and the hatching power of the eggs laid. Minn. Agr. Expt. Sta. Bul. 286:1-12.

Ellis, N. R., D. Miller, H. W. Titus, and T. C. Byerly, 1933. Effect of diet on egg composition. III. The relation of diet to the vitamin B and the vitamin G content of eggs, together with observations on the vitamin A content. J. Nutr. 6:243-262.

Guerrant, N. B., E. Kohler, J. E. Hunter, and R. R. Murphy, 1935. The relationship of the vitamin D intake of the hen to the antirachitic potency of the eggs produced. J. Nutr. 10:167-178.

Guilbert, H. R., 1934. Determination of carotene as a means of estimating the vitamin A value of forage. Ind. and Eng. Chem., Anal. Ed. 6:452-454.

Holmes, A. D., F. Tripp, and P. A. Campbell, 1936a. Vitamin A storage by chickens. Poul. Sci. 15:71-78.

——— 1936b. The vitamin A reserve of embryo and baby chicks. J. Nutr. 11:119-128.

Koenig, M. C., M. M. Kramer, and L. F. Payne, 1935. Vitamin A content of eggs as related to rate of production. Poul. Sci. 14:178-182.

McCoord, A. B. and E. M. Luce-Clausen, 1934. The storage of vitamin A in the liver of the rat. J. Nutr. 7:557-572.

Moore, T., 1931. XXXIV. Vitamin A and carotene. VII. The distribution of vitamin A and carotene in the body of the rat. Biochem. J. 25:275-286.

Morgan, C. L., 1932. Relationship between breaking strength and the percent of egg shell. Poul. Sci. 11:172-175.

Murphy, R. R., J. E. Hunter, and H. C. Knandel, 1934. The vitamin D requirements of growing chicks and laying hens. Penn. Agr. Exp. Sta. Bul. 303:3-24.

Murray, H. A., Jr., 1925. Physiological ontogeny. A. Chicken Embryos. II. Catabolism. Chemical changes in fertile eggs during incubation. Selection of standard methods. J. Gen. Physiol. 9:1-37.

Rosenheim, O. and T. A. Webster, 1927. XVII. The stomach oil of the fulmar petrel (Fulmarus Glacialis). Biochem. J. 21:111-118.

Russell, W. C. and M. W. Taylor, 1935. The relation between the vitamin A and D intake by the hen and the output in eggs. J. Nutr. 10:613-623.

Sherman, H. C. and L. C. Boynton, 1925. Quantitative experiments upon the occurrence and distribution of vitamin A in the body, and the influence of the food. J. Am. Chem. Soc. 47:1646-1653.

Sherwood, R. M. and G. S. Fraps, 1934. The amount of vitamin A potency required by hens for egg production. Texas Agr. Exp. Sta. Bul. 493:5-22.

——— 1935. The vitamin A requirements of hens for egg production. Texas Agr. Exp. Sta. Bul. 514:4-21.

The Pharmacopoeia of the United States, Eleventh Edition, 1936. p. 478. Mack Printing Co., Easton, Pa.

Thomas, B. H. and F. W. Quackenbush, 1933. Effect of diet on the quantity of vitamins A and D occurring in hen eggs. Iowa Agr. Exp. Sta. Rept. p. 27.

The Effect on Yolk Color of Various Ingredients in Poultry Feeds*

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VARIATIONS in egg yolk color are of economic importance to the commercial poultryman. Because of rigid egg grading and candling practices, there is a demand for eggs with uniform-colored yolks for discriminating markets. This, together with the occasional necessity for the poultryman to vary his laying ration, makes it desirable that he know the effect of the various ingredients of his laying ration on the yolk color of the eggs produced. It was the purpose of this investigation to study these relationships.

REVIEW OF LITERATURE

From time to time, during the past three decades, some investigational work pertaining to the problem of egg yolk color has been reported. Dryden (1905) reported the production of highly colored egg yolks by feeding green alfalfa. Before this, it had been frequently observed that yellow corn produced a rich, golden yellow, egg yolk.

Palmer and Kempster (1919) fed various rations to individual hens and determined yolk color by the Lovibond Tintometer. Parker, Gossman, and Lippincott (1925) reported the effect of white corn, yellow corn, and several green feeds on egg yolk color by using a specially constructed color standard.

Stewart, Gans, and Sharp (1932) used a color standard in some candling investigations, but they report no specific yolk color studies. Payne and Wilhelm (1933) used the Munsell color notations in a study "to ascertain the effects on egg yolk color due to the various ingredients of common poultry feeds." White corn, yellow corn, kafir, wheat, oats, yellow milo, alfalfa, and several succulent green feeds were used in this study.

While several different standards of comparison for egg yolk color have been used, most of them have for various reasons, been limited in this use. With the introduction of the Color Roter (Heiman and Carver, 1936), an apparently workable standard of comparison for yolk color was presented.

It was the purpose of this investigation to ascertain the specific effect on yolk color of high levels of various ingredients commonly used in poultry rations.

EXPERIMENTAL

Approximately 200 White Leghorn pullets were placed in laying batteries. At the end of a four-week adjustment period, they were culled on the basis of their previous production, and all birds were given a basal ration to deplete the color of their yolks. This ration consisted of the following ingredients:

Ingredient	*Percent*
Re-cleaned ground white corn	70.0
Dried skimmilk	17.0
Dried brewer's yeast	5.0
Ground wood pulp	3.0
Oyster shell flour	2.0
Steamed bone meal	1.0
Salt	1.0
Concentrated cod liver oil	1.0
Total	100.0

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After a two-week depletion period, all eggs were broken daily and the yolk colors determined by the Color Roter. Again the birds were culled, and the experimental lots of six birds each were arranged as indicated in Figure 1.

In formulating rations for the various lots reported here (Fig. 1), substitutions were made in the basal ration of lots 2 to 7, of 70 percent each of Argentine yellow flint corn, domestic yellow dent corn, barley, wheat, oats, and rye replacing the 70 percent of re-cleaned white corn used in the basal ration. In lots 8 to 15, 17 percent of dehydrated alfalfa leaf meal, green pea meal, soybean oil meal, linseed oil meal, cottonseed oil meal, salmon fish meal, herring fish meal, and copra meal replaced the dried skimmilk. In lots 16, 17, and 18, 3 percent of dried cow manure, dried horse manure, and ground wheat straw were substituted for the ground wood pulp of the basal ration. Fifteen grams per bird of succulent alfalfa, fresh lawn clippings, green curly kale, and fresh carrots were gathered each morning, ground, and fed as a supplement to the basal ration in lots 19, 20, 21, and 22 respectively. Lots 23, 24, and 25 were fed the basal ration (lot 1) and, in addition, the birds were allowed to range on young alfalfa. The birds comprising lot 23 were placed out in individual coops at 7:00 a.m. and remained out until 6:00 p.m. (11 hours of range). Lot 24 was allowed six hours range, from 12:00 m. until 6:00 p.m., while lot 25 was permitted to be out on alfalfa from 4:00 p.m. until 6:00 p.m. Nests, shade, mash, and water were provided while the birds were out on range, and the coops moved to fresh range each day.

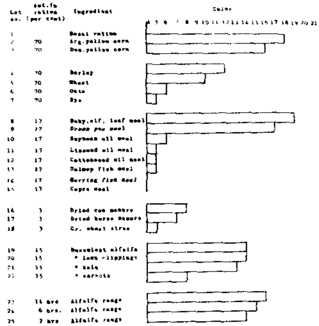

Fig. 1. The effect of various feeds on egg yolk color after prolonged feeding measured by the yolk Color Roter.

The eggs were gathered hourly and held in a humidor. Daily yolk color observations were made, using the yolk color index of Heiman and Carver loc. cit.

RESULTS

A comparison of egg yolk color of lots 1, 2, and 3 shown in Figure 1, indicate 70 percent of Argentine yellow flint corn gave a color of 18, while the same amount of domestic yellow dent corn gave a color reading of 16. Both of these gave more than four times as much color as lot 1, which had an average final reading of 4. Of the other cereal grains, barley gave the most color to the egg yolk with a reading of 12. Wheat gave a reading of 10, while oats and rye had a color of 6 and 5 respectively.

In the group frequently referred to as "protein supplements," lot 8, receiving 17 percent dehydrated alfalfa leaf meal, gave the most highly pigmented yolks. Green pea meal, a product that is available in many sections of the Northwest, gave a color reading of 12. This was somewhat lower than was expected, for this product was bright green in color. Seventeen percent Manchurian soybean oil meal, when fed as the chief pigment-bearing ingredient of the ration, gave slightly more color than an equal amount of cottonseed oil meal or linseed oil meal. Copra meal (cocoanut meal)

gave no increase in yolk color over the basal ration. Salmon fish meal, although a dark red in color, gave a yolk color reading of 5, which was slightly higher than Alaska herring fish meal, which did not increase the basal yolk color.

The cow and horse manures were both produced on the same alfalfa pasture. The dried cow manure gave a color reading of 8, while the dried horse manure gave a reading of 7 as compared to a reading of 14 when the alfalfa was fed. Three percent ground wheat straw gave only a slight increase in yolk color over the basal.

A comparison of succulent feeds furnished at the rate of 15 grams daily per bird, or 3.3 lbs. per 100 birds, produced egg yolk colors of 14 for alfalfa, 14 for lawn clippings, 13 for kale, and 8 for carrots.

The effect of various time intervals on alfalfa range upon egg yolk color showed that 11 hours on alfalfa range results in a yolk color reading of 16. Ranging for six hours gave a color of 15, while range for only two hours resulted in yolk color reading of 14. A comparison of 15 grams fresh alfalfa and two hours on range show that they produce practically the same effect on the yolk color.

SUMMARY

1. The substitution of equal amounts of Argentine yellow flint corn for domestic yellow dent corn results in increased yolk color.

2. Barley produces more yolk color than wheat, oats, or rye.

3. Dehydrated alfalfa leaf meal and green pea meal both contain very appreciable amounts of available pigments.

4. None of the other protein supplements herein reported contain significant quantities of yolk-coloring materials.

5. Succulent alfalfa, fresh lawn clippings, and green curly kale produced essentially the same yolk color, while fresh carrots exerted only a slight effect on yolk color.

6. When the birds were allowed access to alfalfa range for 2, 6, or 11 hours, the resulting yolk color was not materially affected by the length of time on range over the period studied.

BIBLIOGRAPHY

Dryden, J., 1905. Poultry experiments, feeding color into the egg. Utah Agr. Exp. Sta. Bull. 92: 174-175.

Heiman, V. and J. S. Carver, 1935. The Yolk Color Index. U. S. Egg and Poultry Magazine, August, 1935: 40-41.

Heiman, V. and L. A. Wilhelm, 1937. The transmission of xanthophylls in the feeds to the yolk of the hen's egg. (In press.)

Palmer, L. S. and H. L. Kempster, 1919. The influence of specific feeds and certain pigments on the color of the egg yolk and body fat of fowls. Jour. of Biol. Chem. 39:331-337.

Parker, S. L., S. S. Gossman, and W. H. Lippincott, 1925. Studies on egg quality. I. Introductory note on variations in yolk color. Poul. Sci. 5:121-145.

Payne, L. F. and L. A. Wilhelm, 1933. Egg yolk color as affected by certain xanthophyll-bearing feeds. Abstract Poul. Sci. 12:330-331.

Stewart, G. F., A. R. Gans, and P. F. Sharp, 1932. The relation of the color of the yolk to the interior quality as determined by candling and from the opened egg. U. S. Egg and Poultry Magazine. V. 38, No. 10:44-48.

The Measurement of Sexual Maturity and Persistency

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LERNER and Taylor (1937) have presented evidence that the length of the biological laying year cannot be considered an adequate measure of inherent persistency. They have shown that it is conditioned both by the age at first egg and the age at last egg previous to or at the onset of fall molt. Consequently it reflects both sexual maturity and persistency rather than the latter alone. When it is desired to determine persistency separately and when age at first egg is used to describe sexual maturity, age at last egg presents itself as a measure for such purpose.

However, the biological laying year may be determined on the basis of the calendar year rather than on the basis of age. In that case, it may be considered to be conditioned by the date of first egg on the one hand and by the date of last egg on the other. The purpose of this paper is to establish the preference for usage of one of these two scales in experimental work and in breeding practice.

The data for this study were obtained on the same birds which were used for the earlier report (Lerner and Taylor, 1937), consisting of 247 Single Comb White Leghorn pullets hatched at weekly intervals in March and April, 1933, and of 391 pullets of the same variety and similarly hatched at weekly intervals in March and April, 1934. The results of the analysis are presented separately for the two series of birds.

SEXUAL MATURITY

Age at first egg has been used for the measurement of sexual maturity for a number of years and in general has been found satisfactory. The studies on the inheritance of sexual maturity were made using this measure as the definition of this character and considerable evidence as to the nature of the genetic factors involved has been presented by Hays (1924, 1936a), Jull (1934), and Warren (1934). However, it is of interest to determine the feasibility of using date at first egg for a similar purpose. The material available was limited by the comparative shortness of the hatching season, but within the limits of the data available it was possible to derive some pertinent information. No attempt will be made here to review the literature on the subject, this having been very adequately done by Knox, Jull, and Quinn (1935).

Table 1 presents analyses of variance of age at first egg and of date of first egg with respect to the influence of the sires in accounting for a part of the genetic variance, and with respect to environmental influences as expressed by the hatching date. The two analyses for each variable of each series are presented separately because of the disproportionate frequencies obtaining in the different subclasses.

It may be readily seen that so far as the effect of the male parent is concerned, age at first egg presents more significant values of F than does date at first egg. In other words, a greater proportion of the variance observed seems to be of genetic nature in the former than in the latter measure of sexual maturity.

The effect of hatching date on the two

TABLE 1.—*Analysis of variance of age at first egg and of date of first egg*

Source of variance	1933 series			1934 series		
	Degrees of freedom	Age at first egg mean sq.	Date of first egg mean sq.	Degrees of freedom	Age at first egg mean sq.	Date of first egg mean sq.
Between means of sires	18	3688	3675	19	2127	2450
Within means of sires	228	709	1200	371	927	1350
F*	(1.65–2.00)	5.20	3.06	(1.60–1.94)	2.29	1.81
Between hatching dates	6	1729	9400	4	4633	21775
Within hatching dates	240	907	1175	386	948	1175
F*	(2.14–2.88)	1.91	8.00	(2.40–3.37)	4.89	18.53

* The values of F in parentheses correspond to the 1% and the 5% points for F.

measures is different. Date of first egg is significantly influenced by the hatching date in both populations studied, while the effect on age at first egg does not seem to be as definitely established. The F value in this case though highly significant for the 1934 series, falls below the level of significance for the 1933 series.

Thus, age at first egg is a superior measure of sexual maturity both from the standpoint of exhibiting a greater proportion of genetic variance and from the standpoint of greater independence from date of batch within the limits investigated.

PERSISTENCY

Age at last egg and date at last egg have similarly been examined for their usage relative to persistency of laying. Table 2 includes the same type of information as Table 1 with the addition of the analyses involving the effect of age at first egg and date of first egg on the two proposed measures of persistency. So far as the proportion

TABLE 2.—*Analysis of variance of age at last egg and of date of last egg*

Source of variance	1933 series			1934 series		
	Degrees of freedom	Age at last egg mean sq.	Date of last egg mean sq.	Degrees of freedom	Age at last egg mean sq.	Date of last egg mean sq.
Between means of sires	18	4031	4650	19	4230	4550
Within means of sires	228	1895	2175	371	1825	1775
F*	(1.65–2.00)	2.13	2.14	(1.60–1.94)	2.32	2.56
Between hatching dates	6	6048	2525	4	9785	575
Within hatching dates	240	1951	2350	386	1861	1925
F*	(2.14–2.88)	3.10	1.07	(2.40–3.37)	5.26	—†
Between mean ages at first egg	4	2776	5325	5	3624	3825
Within mean ages at first egg	242	2039	2300	385	1920	1900
F*	(2.42–3.40)	1.36	2.32	(2.24–3.06)	1.89	2.01
Between mean dates of first egg	4	3703	3400	4	3291	1650
Within mean dates of first egg	242	2024	2350	386	1928	1925
F*	(2.42–3.40)	1.83	1.45	(2.40–3.37)	1.71	—†

* The values of F in parentheses correspond to the 1% and the 5% points for P.
† Variance greater within than between means.

of genetic variance is concerned, the age and the date measures appear to be equivalent, since the F values are practically the same for both in each of the series.

The hatching date, however, shows a differential effect. While it has a highly significant influence on age at last egg, the variance within hatching dates of date at last egg is nearly equal to the variance between hatching dates in the 1933 series and exceeds it in the 1934 series.

Neither age at first egg nor date at first egg have any effect on either of the two persistency measures, as shown by the F values, which agrees with the conclusions presented earlier (Lerner and Taylor, 1937) with respect to the independence of age at last egg from the age at first egg.

Thus, within the hatching season studied date of last egg as a measure of persistency seems somewhat superior to age at last egg. However, since age has a very firm genetic basis so far as sexual maturity is concerned, age at last egg may, perhaps, be used as a measurement of persistency, especially if it is corrected for hatching date.

In general, both of these measures have advantages and disadvantages from the practical point of view in application to problems of poultry breeding. Date of last egg can be obtained at a glance from the trapnest record. Selection for persistency can then be made readily by choosing a date of last egg as the standard. The more severe the standard desired the later this date would be. This method is particularly applicable if persistency is inherited on a multifactorial basis. If, on the other hand, only one factor is involved, the distribution of dates of last egg in a flock would be bimodal and the determination of the critical dates can be readily made by a dissection of the frequency distribution curve, as Hays (1936b) has done with the length of biological laying year and with sexual maturity (1936a).

Age at last egg, on the other hand, has the advantage of being on an equivalent scale to the measurement used for sexual maturity. No coding would be required for it in statistical work and determination of the length of biological laying year, if used for estimation of annual rate or other constants, can be readily made from these two values.

Statistical assistance under the auspices of the WPA is acknowledged.

CONCLUSIONS

1. Age at first egg is superior to date at first egg as a measure of sexual maturity.

2. While date of last egg is a better measure of persistency than age at last egg, the latter may be used, if corrected for hatching date.

REFERENCES

Hays, F. A., 1924. Inbreeding the Rhode Island Red fowl with special reference to winter egg production. Am. Nat. 58:43-59.

——— 1936a. Inheritance of sexual maturity in Rhode Island Reds. Papers 6th World's Poultry Congress, Berlin-Leipzig. 2:34-38.

——— 1936b. Studies on the inheritance of persistency. Genetics 21:519-524.

Jull, M. A., 1934. The inheritance of sexual maturity, rate, and persistence of laying in the domestic fowl. Poul. Sci. 13:286-289.

Knox, C. W., M. A. Jull, and J. P. Quinn, 1935. Correlation studies of egg production and possible genetic interpretations. J. Agric. Res. 50:573-589.

Lerner, I. M. and L. W. Taylor, 1937. The spurious nature of the linkage between length of laying year and sexual maturity in the fowl. Am. Nat. (in press).

Warren, D. C., 1934. Inheritance of age at sexual maturity in the domestic fowl. Genetics 19:600-617.

Spotting and Striping in Exhibition Classes of Rhode Island Red and New Hampshire Baby Chicks

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IN A PREVIOUS paper (Byerly and Quinn, 1936) it was shown that 47.5 percent of 1,102 Rhode Island Red chicks hatched at the National Agricultural Research Center, Beltsville, Maryland, carried black spotting on the head or black striping on either head or body, also that 42.1 percent of 663 chicks from an excellent standard bred flock carried spotting or striping, indicating that these characteristics may be rather widely distributed among Rhode Island Red flocks. It was further shown that 84.9 percent of the striped or spotted Beltsville chicks were females, whereas 77.8 percent of the non-spotted, non-striped chicks were males.

Several breeders and hatcherymen have questioned the extent of the distribution of the characteristics mentioned above. They have also pointed out that little melanic pigment (black spotting and striping) appears in the Rhode Island Red Chicks shown in commercial and standard bred exhibition classes. It was the opinion of the authors that exhibitors of baby chicks practiced considerable selection, by choosing non-spotted and non-striped chicks for these exhibition classes, in order to obtain a uniform appearance in each lot, and that such selection would probably result in a preponderance of male chicks. In order to verify or disprove this opinion, observations on the down color and sexes of Rhode Island Red and New Hampshire chicks were made at Northeastern Poultry Producers Exposition in November, 1936.

Of the 650 chicks observed in the 26 entries, only 24.9 percent of the Rhode Island Red chicks and 13.3 percent of the New Hampshires showed any melanic pigment in down color. One hundred of the 650, or 15.4 percent, carried one or more black spots on the head but only 21 chicks, or 3.2 percent, showed any striping at all. Of the striped chicks, 12, or 1.8 percent, carried a head or shoulder stripe, and 9, or about 1.4 percent, carried a head and body stripe.

No single individual chick, however, showed a full complement of striping in head and body such as is found in standard bred Brown Leghorn chicks, and which has been occasionally observed in individual Rhode Island Red chicks at Beltsville. These observations, therefore, showed that a smaller percentage of exhibition chicks carried melanic pigment than was previously observed in the Beltsville investigations. Furthermore, the exhibition chicks which did carry melanic pigment had less than is commonly observed. It appeared, than, that exhibitors, in seeking a uniform red down color in each lot, had chosen non-spotted and non-striped chicks in preference to chicks carrying black pigment in down.

Such a method of selection would appear to affect the sex ratio, increasing the number of males and decreasing the number of

females. A few lots of chicks were sexed to determine whether or not there was an actual preponderance of male chicks. Five lots of Rhode Island Red chicks and five lots of New Hampshires were chosen for this purpose, the sexing being accomplished by dissection. The 10 lots of chicks were from 10 different breeders and appeared to be representative of the whole group of 26 entries, the percentage of spotted and striped chicks being 18.6 percent for the whole group, and 19.4 percent for the chicks to be sexed. The results are given in Table 1.

Table 1.—*Sexes in exhibition classes of Rhode Island Red and New Hampshire chicks, classified according to down markings.*

Lot no.	Non-spot		Spot or spots and striping	
	Males	Females	Males	Females
Rhode Island Reds				
1	13	9	0	3
2	9	10	0	5
3	14	4	1	5
4	11	6	3	4
5	8	1	3	13
Total	55	30	7	30
New Hampshires				
6	13	8	1	2
7	19	5	1	0
8	12	12	0	1
9	16	5	1	1
10	17	3	0	3
Total	77	33	3	7
Grand total	132	63	10	37

The selection of these exhibition chicks for uniform red down color apparently resulted in choosing more males than females, the ratio being 142 to 100. Most of the non-spotted chicks, or 67.7 percent, were males, and most of the spotted and striped chicks, or 78.8 percent, were females. These observations of selected exhibition chicks from 10 different breeders appear to approximate the results of the Beltsville observations on unselected Rhode Island Red chicks, among which it was found that 77.8 percent of the non-spotted, non-striped chicks were males, and 84.9 percent of the black spotted and striped chicks were females. The percentage of spotted and striped individuals among the sexed exhibition chicks was 19.4 percent, compared to 47.5 percent and 42.1 percent among the unselected Beltsville chicks.

In the spotted chick group, the classification of chicks was based on black spotting alone, all spots of other colors, being disregarded. A few of the sexed chicks were found to carry a red spot or spots on the head. These were classed as non-spots, five being males and eight females. Observations at Beltsville on 938 Rhode Island Red chicks from 11 breeding pens showed that 57 had red spots on the head, 29 being males and 28 females. The presence of a red spot or spots, therefore, probably has no significance as to sex among the exhibition chicks observed. It is interesting to note,

however, that among the 57 red head-spotted chicks observed at Beltsville there were 13 which had two red spots or more, three being males and 10 females.

It has been suggested that a preponderance of males over females in the exhibition classes might mean that the selection of newly hatched chicks was based on individual chick weights, the larger and more vigorous chicks being males, and one or two cases have come to the authors' attention where it was claimed that selection of newly hatched chicks for size had resulted in a highly disproportionate number of males being reared. There is no experimental evidence for such a contention, however, as several investigators have shown quite definitely that, although egg weight and chick weight were correlated, egg weight bears no relation to sex ratio. Jull and Quinn (1925) found no significant difference between the weights of male and female chicks at hatching time, and Jull and Heywang (1930) found that the ratio of chick weight to initial egg weight is independent of the sex of the chick.

The New Hampshire chicks, as a group, appeared to show less melanic pigment than the Rhode Island Reds, as stated previously. Whether this was due to more careful selection, or whether most New Hampshire flocks carry somewhat less pigment than Rhode Island Reds, was not known, as there had been no opportunity to examine the down color of chicks from representative New Hampshire flocks. In the five lots of New Hampshires shown in the broiler production class, however, where the scores were made on rate of gain in weight, more melanic pigment was observed in the chicks than in the New Hampshire exhibition chicks shown in Table 1. Three of the five lots had one or more chicks with incomplete body striping and black head spotting or striping. One of these three lots had 18 non-spotted chicks, six chicks with two black spots on the head, and one chick with four dark incomplete body stripes and two black spots on the head. However, some of the Rhode Island Red chicks in the broiler production class, and in other classes, showed more black pigment than this lot of New Hampshires.

Since it appeared that the New Hampshire chicks in the exhibition classes carried less melanic pigment than the Rhode Island Reds, observations were made on the down color of New Hampshire chicks from a nearby Maryland flock. Of 296 New Hampshire chicks examined, 29.4 percent carried black spotting and striping. This percentage of black in down is more than twice as much as that shown by the New Hampshire chicks in the exhibition classes but is much less than that shown by unselected Rhode Island Red chicks. If these New Hampshire chicks were representative of the breed it would appear that New Hampshire chicks do carry considerably less melanic pigment than Rhode Island Reds. The New Hampshire chicks observed came directly from the flock of a nationally known New Hampshire breeder. There may be a good deal of variation in the down color characteristics of chicks from New Hampshire flocks, however.

The authors wish to express ther appreciation to Mr. R. E. Jones, chairman of the Baby Chick Committee of the Northeastern Poultry Producers Exposition and to his assistants, Mr. Wheeler and Mr. Johnson, for permission to make individual observations of chicks and for their generous coöperation.

SUMMARY

Observations on the down color of 650 Rhode Island Red and New Hampshire chicks in 26 exhibition entries showed that only 24.9 percent of the Rhode Island Reds and 13.3 percent of the New Hampshires carried black spotting and striping, whereas 47.5 percent of the Beltsville Rhode Island Red chicks and 42.1 percent of the chicks

from another standard bred Rhode Island Red flock carried melanic pigment. Exhibitors had apparently chosen non-spotted and non-striped chicks in preference to chicks carrying black pigment in down.

This selection of uniformity of red down color resulted in a disproportionate sex ratio, 142 of the sexed chicks being males, and 100 females.

Among the sexed chicks, the preponderance of males in the non-spotted, non-striped chicks, and the preponderance of females in the black spotted and striped group appear to coincide with previous observations in chicks from the Beltsville flock, although the 10 lots of sexed chicks were selected chicks which came from 10 different breeders. Among the exhibition chicks, 67.7 percent of the non-spotted, non-striped chicks were males, and 78.8 percent of the black spotted and striped chicks were females. In the Beltsville observations on unselected chicks, 77.8 percent of the non-spotted, non-striped chicks were males and 84.9 percent of the black spotted and striped chicks were females.

The New Hampshire chicks observed in the exhibition classes carried considerably less black pigment in down than the Rhode Island Red chicks observed in the exhibition classes. The New Hampshire chicks from a nearby Maryland flock also carried less melanic pigment than the Rhode Island Red chicks observed at Beltsville and elsewhere.

References

Byerly, T. C. and J. P. Quinn, 1936. Sexual dimorphism in Single Comb Rhode Island Red down color. Jour. Hered. 27:319.

Jull, M. A. and J. P. Quinn, 1925. The relationship between the weight of eggs and the weight of chicks according to sex. Jour Agr. Res. 31:233.

———, and B. W. Heywang, 1930. Yolk assimilation during the embryonic development of the chick. Poul. Sci. 9:195.

Kentucky Bluegrass in All-Mash Rations*

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THIS experiment was planned in order to give a more definite answer to the frequently asked question, "Can dried Kentucky bluegrass be used in an all-mash ration for battery-brooder raised chicks?"

In a previous publication[1] experiments were described, the results of which indicated that neither irradiated nor unirradiated fresh bluegrass fed *ad libitum* to White Leghorn chicks, will replace cod liver oil as a source of vitamin D in the prevention of rickets, but that irradiated dried bluegrass will. Dried bluegrass was not a proper substitute for dried skimmilk on the basis of equal quantity of protein. Chicks raised on grass ranges probably receive the necessary antirachitic vitamins from direct sunlight and not from the green herbage.

Experiments have further shown that the average daily consumption of fresh bluegrass, per chick, during the first three weeks was approximately 3 grams, and for the next three weeks, from 4 to 5 grams. An adult White Leghorn hen or cockerel will consume daily an average of 10 grams of fresh bluegrass.

Three lots, each containing 30 one-day-old White Leghorn chicks, were placed in separate compartments in an indoor battery-brooder. A control ration consisting of yellow corn 64, mixed wheat feed 18, dried skimmilk 14, tricalcium phosphate 2, so-

* The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

[1] Proceedings of the 6th World Poultry Congress, Leipzig, Germany, Vol. 1, Section 2, page 240, 1936.

Table 1.—*Composition of mashes fed. Percent*

Lot No.	Ground yellow corn	Mixed wheat feed	Dried skim-milk	Air-dried blue-grass	Tri-calcium phosphate	Sodium Chloride	Sardine oil
1	64	18	14	—	2	1	1
2	65	0	14	17	2	1	1
3	62	0	7	27	2	1	1

Table 2.—*Analyses of mashes fed to each lot. Percent*

Lot No.	Protein	Fat	Fiber	Ash	Ca	P
1	15.0	4.3	2.9	5.6	.8	.8
2	14.7	5.0	6.9	6.5	1.2	.8
3	14.9	5.2	14.8	9.8	1.4	.9
Air-dried bluegrass	17.2	4.0	20.1	7.5	.7	.5
Mixed wheat feed	16.2	4.4	8.3	5.2	.1	1.0

dium chloride 1, and sardine oil 1, was fed. This ration had repeatedly given good growth with chicks raised under these conditions. Lot 2 was fed the same ration except that 17 parts of air-dried Kentucky bluegrass was substituted for the 18 parts of mixed wheat feed which was on an equal protein basis. The mash fed lot 3 was the same as lot 2, except 7.5 parts of the dried skimmilk was substituted by air-dried bluegrass on an equal protein basis, making this ration contain 27 parts of dried bluegrass. These three rations contained approximately 15 percent protein.

The composition and analyses of the mashes fed are given in Tables 1 and 2, respectively.

Concerning the feed consumption per chick, it was found that lot 1 consumed at the normal rate for battery-brooder raised chicks, and lots 2 and 3 consumed smaller quantities of mash in the order given. This was due to the bulkiness of the mash containing the air-dried bluegrass. A comparison of the volume-weight ratio shows that 1,000 c.c. of ground air-dried bluegrass, mixed wheat feed and dried skimmilk weighed, respectively, 140, 323, and 317 grams.

The number of chicks alive after 10 weeks in lots 1, 2, and 3 were 29, 21, and 10, respectively, and their respective weights were 552, 345, and 329 grams. No slipped tendons occurred.

Because of the bulkiness of air-dried bluegrass, due to the high fiber content, it is not a satisfactory ingredient in all-mash rations for growing chicks. Bluegrass should be fed *ad libitum* as a succulent feed.

Studies on Incubator Hygiene

IX. The Disinfecting Properties of Formaldehyde for Hemophilic Bacteria Associated with Avian Coryza, the Filtrable Viruses of Infectious Laryngotracheitis and Infectious Bronchitis

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DURING the past four years the occurrence of an acute, highly contagious and fatal respiratory syndrome in chicks three days to 10 weeks of age has been responsible for many inquiries regarding the nature as well as effective methods of prevention and control. Natural outbreaks coming to the attention of the Illinois Agricultural Experiment Station have been observed in broiler-feeding plants as well as in chicks on farms. In some outbreaks studied, the infection apparently originated in commercial hatcheries, while in others exposure during transit or following arrival at the farm or plant could not be excluded.

Methods of control suggested to owners of infected chicks for the suppression of acute respiratory diseases place emphasis on the value of sanitary measures including complete isolation or destruction of clinically affected, exposed, and recovered chicks together with cleaning and disinfection of quarters and equipment. In fact, the value of these measures was stressed in various outbreaks pending a definite diagnosis of the disease or diseases.

HYGIENIC MEASURES

The value of hygienic measures as appraised by the recession or the disappearance of the disease in natural outbreaks apparently justified the methods suggested, notwithstanding the recurrence of the disease or a similar disease in newly-purchased chicks on certain disinfected premises. In an explanation of the recurrence of the disease or diseases, a variety of factors are presented for consideration, including the possibility of improper cleaning or inefficient disinfection of premises, failure of the fumigant in amounts employed together with a carrier feature of the diseases and the occurrence of more than one respiratory disease characterized by similar symptoms and gross lesions.

DISINFECTING PROPERTIES OF FORMALDEHYDE

To appraise the value of cleaning and disinfection of premises in the control of respiratory diseases of chicks, an attempt was made to measure the value of formaldehyde as (1) a bactericidal agent for hemophilic bacteria of avian coryza, and (2) its virucidal properties for the viruses of avian laryngotracheitis, and avian bronchitis.[1] The experimental data presented herewith offer critical evidence in support of the germicidal as well as the virucidal efficiency of formaldehyde for two strains of hemophilic bacteria from outbreaks of avian coryza, two strains of avian laryngotracheitis virus, and one strain of avian virus

bronchitis.* These viruses were propagated upon developing chicken eggs according to the method described by Brandly.[2] Within limitations it appears that this disinfectant is of value in the suppression and control of spontaneous outbreaks of these respiratory diseases with special reference to their destruction in a forced-draft incubator.

The germicidal and virucidal efficiency of formaldehyde in a forced-draft incubator† was previously reported for *S. pullorum*, *S. aertrycke*, *S. anatum*, *Salmonella sp.* from quail, and fowl pox virus.[3-6] The amounts of formaldehyde employed proved noninjurious to hatching chicks. *S. pullorum* on the feet and down of chicks was destroyed in an average of 57 and 69 minutes when released with potassium permanganate (25 c.c. of formalin per 100 cubic feet) and in an average of 69 and 84 minutes when released from cheesecloth at the rate of 16 2/3 c.c. of formalin per 100 cubic feet. Stated in another way, 100 c.c. of formalin released directly from cheesecloth possessed a germicidal efficiency against *S. pullorum* in a forced-draft incubator comparable to 150 c.c. of formalin when released with potassium permanganate. A viability range for *S. pullorum* on the feet and down of chicks following formaldehyde fumigation was noted up to 120 minutes in the potassium permanganate release, while a range of viability up to 150 minutes was occasionally noted in formaldehyde fumigation when the release was from cheesecloth.

Fowl pox virus survived formaldehyde fumigation in forced-draft incubator 30 minutes with an occasional range of survivability of 45 and 50 minutes but fowl pox virus was consistently destroyed after a 90-minute period of fumigation. Formaldehyde was released from cheesecloth (16 2/3 c.c. of formalin per 100 cubic feet). Cotton squares as well as the feet and down of day-old chicks were immersed in a 1 percent aqueous suspension of the powdered pox scabs. The moist pox virus contaminated squares and chicks were placed in the hatching trays and subjected to formaldehyde. The results indicated the virucidal properties of formaldehyde for pox virus in amounts recommended in forced-draft incubators for the suppression and control of incubator-disseminated pullorum disease although the average period of survivability of fowl pox virus as well as the range of survivability following fumigation is somewhat shorter than that of *S. pullorum*.

FORMALDEHYDE FUMIGATION OF HEMOPHILIC BACTERIA FROM AVIAN CORYZA

Technic: Sterile absorbent cotton squares 1 x 1 x 1/16 in. were immersed in a physiological salt suspension of two separate cultures of avian hemophilic bacteria designated 1 and 2. The density of the suspension was comparable to McFarland nephelometer tube No. 1. The artificially contaminated squares were exposed in a false window on the side of a Smith Model 30 Incubator, near the hatching trays.‡ For fumigation 100 c.c. of formalin were released by the cheesecloth method. Squares were removed at intervals (Table 1) and cultured on 10 percent horse blood agar plates and in 10 percent horse blood serum broth. The cultures were made by pushing the cotton square to the end of the wire holder and streaking heavily on a blood agar plate. The cotton was then placed into

* Strain of virus made available through the courtesy of Dr. F. R. Beaudette, of New Jersey Agricultural Experiment Station.

† The relative humidity of the incubator during the course of these fumigations was approximately 68 percent (a wet bulb reading of 90°F. when the dry bulb reading was 100°F.).

‡ A Smith Model 30 forced-draft incubator was employed in these studies, and the eggs for hatching purposes were procured with funds provided by the Smith Incubator Company.

a tube of 10 percent blood nutrient broth.

Plates were read after 48 and 96 hours' incubation at 37°C. The tubes inoculated in the above manner were incubated 48 hours at 37°C., then streaked on blood agar plates. Media of the type employed for culturing the fumigated swabs were inoculated with unfumigated swabs to serve as controls.

Results: The survival time of two hemophiles during formaldehyde fumigation is recorded in Table 1. In the first experiment hemophile 1 survived 15 and 30 minutes' fumigation as judged by the cultural results. During the fumigation period 20,000 eggs were in the incubator. The remaining tests were conducted with 4,400 eggs in the incubator. In a second fumigation this organism survived only 15 minutes as determined by plate cultures. Broth cultures were contaminated. The same results were obtained in a third fumigation. In a fourth, the survival time was 15 minutes by plate culture and 30 minutes by broth culture. In a fifth, a few hemophile colonies appeared after 30 minutes on plate cultures and as long as 60 minutes in broth cultures.

TABLE 1.—*Formaldehyde fumigation of avian hemophilic bacteria*

Fumigation	Hemophile	No. of eggs in incubator	Cotton squares cultured, minutes							No fumigation control
			15	30	60	90	120	150	180	
1.	1	20,000	+	+	−	−	−	−	−	+
2.	1	4,400	+	−	−	−	−	−	−	+
3.	1	4,400	+	±	−	−	−	−	−	+
4.	1	4,400	+	+	−	−	−	−	−	+
5.	1	4,400	+	±	−	−	−	−	−	+
6.	2	20,000	+	+	−	−	−	−	−	+
7.	2	4,400	+	±	−	−	−	−	−	+
8.	2	4,400	+	+	−	−	−	−	−	+
9.	2	4,400	+	+	−	−	−	−	−	+
10.	2	4,400	+	+	−	−	−	−	−	+

+ = Micro-organisms still viable
± = A few colonies still viable
− = No growth

Hemophile 2 survived 30 minutes as judged by cultured results on agar plates and 45 minutes in broth. At the time of this fumigation 20,000 eggs were in the incubator and in the following fumigations 4,400 eggs were undergoing incubation. In a second fumigation the viability of this organism was demonstrated after 15 minutes in blood agar plates but no growth was obtained in broth after fumigation at any period tested. In a third fumigation the hemophile 2 survived 30 minutes on agar plate as judged by the results of cultures and was non-viable when tested in broth. In a fourth fumigation blood agar plates yielded growth of hemophile 2 when inoculated with contaminated cotton squares after 30 minutes' fumigation but broth cultures were viable only after 15 minutes. In a fifth fumigation the organism survived 30 minutes (blood agar plates) and 60 minutes as determined by the broth method.

The results of five separate fumigations with each of two hemophilic bacteria suggest that formaldehyde released by the cheesecloth method (100 c.c. of formalin or 16 2/3 formalin per 100 cubic feet) destroyed the hemophiles 1 and 2 in a clean forced-draft incubator in 60 minutes, though both cultures survived 30 minutes' fumigation in a majority of cases.

FORMALDEHYDE FUMIGATION OF MOIST LARYNGOTRACHEITIS VIRUS

Technic: Preceding formaldehyde fumigation studies on laryngotracheitis virus,

preliminary observations were made on the effect of drying a 10 percent suspension egg propagated virus in tryptone broth. The virus suspension was placed on one cotton square, while two eggs incubated 12 days were dipped in the tryptone virus suspension. One contaminated cotton square and the two virus contaminated eggs were incubated at 37°C. for one hour. The dried cotton square was promptly immersed in 1.5 c.c. sterile tryptone broth and .25 c.c. was instilled into the trachea of two healthy chicks. Both chicks developed typical symptoms and lesions of laryngotracheitis within four days. The virus-dipped eggs following removal from incubator were washed with 2.5 c.c. tryptone broth, and approximately 1 c.c. was administered intratracheally to two separate healthy chicks. The chicks remained apparently healthy. In proving the virulency of the 10 percent virus suspension in tryptone broth, approximately 25 c.c. was left at room temperature for four hours in a sterile mortar. Two healthy chicks were exposed by administering .25 c.c. intratracheally. Both chicks developed laryngotracheitis in four days.

Further observations of the viability of the virus were made by immersing sterile cotton squares and four eggs (incubated 12 days) in a 10 percent tryptone virus suspension as employed in tests to determine the effect of drying on the virus of laryngotracheitis. The freshly contaminated cotton squares and eggs were placed in a forced-draft incubator and subjected to formaldehyde fumigation released from cheesecloth at the rate of 16 2/3 c.c. of formalin per 100 cubic feet. Following fumigation for 30 and 60 minutes, respectively, the cotton squares and eggs were washed in 1.5 c.c. to 2.5 c.c. of sterile tryptone broth, respectively. Two healthy chicks were exposed by intralaryngeal instillation of the resuspended content of the fumigated cotton squares and eggs, respectively. The exposed chicks showed no symptoms of laryngotracheitis over a period of 16 days.

The virucidal properties of formaldehyde were further observed with two separate strains of laryngotracheitis virus. Both strains of virus were propagated on the extraembryonic membranes of chick embryos. The virus-enriched membranes were harvested aseptically and ground in sterile mortars and suspended in tryptone broth and distilled water.

A series of cotton squares, as employed in fumigation of hemophilic bacteria, were immersed in a 5 percent tryptone broth virus suspension and another series of cotton squares were treated in a similar manner in a 5 percent distilled water suspension. The tryptone broth virus was kept for 28 hours at — 4°C. before being employed in fumigation tests, while the distilled water virus suspension was promptly subjected to formaldehyde fumigation. The artificially contaminated cotton squares were fumigated at 30, 60, and 90 minutes in forced-draft incubator with formaldehyde released from cheesecloth in the usual concentration.

Following the fumigation, the contaminated cotton squares were washed in 1 to 2 c.c. of sterile tryptone broth and another series of cotton squares were washed in sterile distilled water. The viability of the virus suspended in tryptone broth and distilled water following fumigation was determined by instilling .25 c.c. intratracheally in healthy chicks. Controls to establish the virulency of the two viruses before fumigation included intralaryngeal instillation of two chicks with the unfumigated viruses (see Table 2).

Results: In a series of two fumigations with separate egg propagated avian laryngotracheitis viruses suspended in tryptone broth and distilled water, respectively, both viruses proved non-infective after 30, 60, and 90 minutes. The control or unfumigated

TABLE 2.—*Formaldehyde fumigation of aqueous and broth suspensions of dried avian laryngotracheitis virus*

Laryngotracheitis Virus*	Vehicle	Fumigation Results (See Table 3)			
		30 min.	60 min.	90 min.	C
1. Tryptone broth suspension	Cotton squares	–	–	–	+
2. Distilled water	Cotton squares	–	–	–	+

* 5 percent
min. = minutes
– = non-infective for chicks; + = infective for chicks

viruses of avian laryngotracheitis in broth and distilled water proved viable (see Table 2).

FUMIGATION OF DRIED LARYNGOTRACHEITIS VIRUS

Technic: A single strain of finely ground egg propagated laryngotracheitis virus in the form of dried infected chorio-allantoic membrane* was sprinkled on sterile cotton squares, and subjected to formaldehyde fumigation in a forced-draft incubator for varying periods of time. Sixteen and two-thirds c.c. formalin per 100 cubic feet were released from cheesecloth. Following the release of formaldehyde, the artificially contaminated cotton squares were removed from the incubator at 30, 60 and 90 minutes. The fumigated cotton squares were then washed in sufficient distilled water to make a 10 percent suspension of the powdered virus. The fumigated virus suspensions in distilled water were then introduced intratracheally into healthy chicks. Each fumigated virus, as well as a composite of the unfumigated virus, was administered in doses of .25 c.c. to two healthy chicks. This protocol was conducted in duplicate with the same virus and technic at an interval of 21 days. Unfumigated controls were used to establish the virulency of the virus.

Results: In the first fumigation at 30, 60, and 90 minutes, evidence was obtained to suggest that formaldehyde in amounts employed did not destroy the dried ground virus of laryngotracheitis (see Table 2). Similar results were obtained in a second fumigation of the dried ground virus with similar amounts of formaldehyde (see Table 2).

FUMIGATION OF AVIAN BRONCHITIS VIRUS

Technic: Finely ground egg propagated avian bronchitis virus (.1 gram) was sprinkled on cotton squares, and subjected to formaldehyde fumigation in a forced-draft incubator. Sixteen and two-thirds c.c. of formalin were released from cheesecloth per 100 cubic feet of space. The contaminated cotton squares were removed at 30-, 60-, and 90-minute intervals. Following

* Fresh tissue dried over P_2O_5 in vacuo at 0 to – 4°C.

TABLE 3.—*Fumigation of dried laryngotracheitis virus**

Fumigation	Vehicle	Fumigation Results			
		30 min.	60 min.	90 min.	C
A	Cotton squares	+	+	+	+
B	Cotton squares	+	+	+	+

* 10 percent
+ = infective for chicks

TABLE 4.—*Fumigation of avian bronchitis virus*

Bronchitis Virus	Vehicle	Fumigation Results			
		30 min.	60 min.	90 min.	C
Powdered infective egg membranes	Cotton squares	+	−	−	+

− = non-infective for chicks; + = infective for chicks.

fumigation each treated virus contaminated cotton square was immersed separately in 1 c.c. of sterile distilled water and .25 c.c. of the suspension was administered into the trachea of each of two healthy chicks. An unfumigated 10 percent suspension of the dried virus in distilled water proved infective when administered to two healthy chicks in amounts of .25 c.c. intratracheally.

Results: The dried bronchitis virus following 30 minutes' fumigation after being resuspended in distilled water proved infective to one of two healthy exposed chicks, while the two chicks exposed to the 60- and to the 90-minute fumigated virus intratracheally remained healthy (see Table 4).

SUMMARY

The results of subjecting two hemophilic bacteria associated with avian coryza, two strains of laryngotracheitis virus, and one strain of bronchitis virus to formalin in amounts of 16 2/3 c.c. per 100 cubic feet, in a forced-draft incubator, with relative humidity of approximately 68 percent (wet bulb reading of 90°F. to dry bulb of 100°F.) tends to support the value of fumigation procedures previously recommended for suppressing dissemination of pullorum disease in forced-draft incubators, to-wit:

a. Two hemophilic bacteria from cases of avian coryza were non-viable after 60 minutes in five fumigations.

b. Laryngotracheitis virus in aqueous and broth suspensions was non-viable after 30 minutes in one fumigation, while the dried finely-powdered virus was viable in two fumigations after 90 minutes.

c. Avian bronchitis virus in dried finely-powdered form was non-viable after 60 minutes' fumigation.

On the basis of preliminary results, fumigation procedures recommended for the prevention of incubator disseminated pullorum disease may be effective in destroying certain respiratory infections that have gained entrance to the incubator. However, it is apparent that dried laryngotracheitis virus may survive 90 fumigation minutes emphasizing the necessity of thorough cleaning in successful fumigation of a forced-draft incubator.

REFERENCES

1. Beach, J. R., and O. W. Schalm, 1935. A filtrable virus distinct from that of laryngotracheitis, the cause of a respiratory disease in chicks, Poul. Sci. 14, 5:314.
2. Brandly, C. A., 1935. Some studies on infectious laryngotracheitis. The continued propagation of the virus upon the chorio-allantoic membrane of the hen's egg, Jour. Inf. Dis. 57:201-206.
3. Graham, Robert, and V. M. Michael, 1932. Studies on incubator hygiene, I. Poul. Sci., 11:110-116.
4. ———, 1932 b. Studies on incubator hygiene. II. Poul. Sci. 11:197-207.
5. ———, and E. H. Barger, 1936. Studies on incubator hygiene, IV. Poul. Sci. 15:48-52.
6. ———, and V. M. Michael, 1936. Studies on incubator hygiene, VIII. Poul. Sci. 15:494-495.

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The Relative Flavin (Vitamin G)[1] Content of Dried Skimmilk, Dried Whey, and Dried Buttermilk*

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THE need for vitamin G by poultry, particularly during the growing and breeding period in the life cycle, has been established by Hauge and Carrick (1926), Norris et al (1933-36), Bethke (1933), Heiman (1935), and others. In poultry feeding, the flavin requirement may be met by feeds from several sources, among which are dairy by-products, yeast products, dry or succulent green feeds, and, under some conditions, by certain packing house and marine products. Dairy by-products especially are used extensively to supply flavin.

Heiman, loc. cit., demonstrated the differences which exist in the relative vitamin-G content of various dairy products, particular emphasis having been given to dried skimmilk and dried whey, which are extensively used as poultry feeds.

The principal purpose of the study reported here was to determine the relative flavin content of dried buttermilk, another dairy by-product, often used in poultry feeding.

PROCEDURE

A group of 500 day-old White Leghorn cockerels was placed in battery brooders

[1] The use of the term flavin is reserved here to identify the growth-promoting phase of the vitamin-G complex.

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TABLE 1.—*Composition of experimental rations**

Groups	1, 6, 11	2, 7, 12	3, 8, 13	4, 9, 14	5, 10, 15
Ingredient	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Ground yellow corn meal	63.2	53.9	61.3	60.4	61.2
Wheat flour middlings	20.0	20.0	20.0	20.0	20.0
White wheat bran	5.0	5.0	5.0	5.0	5.0
Purified casein	8.1	7.7	7.2	8.1	7.3
Dried neutralized whey	—	10.0	—	3.0	—
Dried skimmilk	—	—	3.0	—	—
Dried sweet cream buttermilk	—	—	—	—	3.0
Ground oyster shell	0.2	0.6	0.6	0.6	0.4
Steamed bone meal	2.7	2.0	2.1	2.1	2.3
Salt	0.5	0.5	0.5	0.5	0.5
Concentrated cod liver oil	0.3	0.3	0.3	0.3	0.3
Total	100.0	100.0	100.0	100.0	100.0

* The chemical composition of these rations was 18.0 percent protein, 1.0 percent calcium, and 0.8 percent phosphorous.

and fed the negative control ration described in Table 1. This control ration was complete in all the known nutrients required by chicks except flavin. The reserve supply of flavin in the chicks was depleted by feeding this ration for two weeks as previously described by Wilgus et al (1935) and Davis and Norris (1936).

At the end of two weeks, individual chicks were selected at random and weighed. A distribution curve was developed from these weights and only chicks weighing within ±5 grams of the mean were used for the experiment. These selected chicks were divided into 15 groups of 15 chicks each so that an equal number of chicks per group was selected from each of the weight classes used. Such grouping resulted in comparable samples on the basis of body weight at the beginning of the experiment.

Following this selection, each chick was wing banded and the groups were placed in similar pens of battery brooders. The five experimental groups were then fed the rations. By this procedure each of the rations was fed to the three separate groups of chicks. The purpose of replicating groups was to check the accuracy of the results obtained.

TABLE 2.—*Summary of body weight, mortality, and nutritional paralysis*

Group	Variable in diet	Average weight at 6 weeks by groups	Average weight at 6 weeks by rations	Mortality	Paralysis during 4th, 5th, and 6th weeks
		grams	*grams*	*no. of cases*	*no. of cases*
1		120		3	8
6	negative control	123	121	3	10
11		119		3	10
2		185		0	13
7	3% neutralized dried whey	176	180	1	12
12		177		1	13
3		222		3	9
8	3% dried sweet cream buttermilk	228	223	2	10
13		219		0	14
4		174		1	14
9	3% dried skimmilk	174	174	1	13
14		173		1	12
5	10% neutralized dried whey	330	326	0	0
10	(positive control)	326		0	0
15		323		2	0
L.D.*			7		

* The least difference between any two means required to give odds of 19:1. The method of analysis corresponds to Example 3, and the interpretation is based on Example 4 of Snedecor (1934).

The chicks were weighed individually at weekly intervals during the following four-week experimental period and observations on the occurrence and severity of nutritional paralysis were made and recorded.

The milk products used in the experiment were dried skimmilk, dried neutralized whey, and dried sweet cream buttermilk. These materials were prepared from similar supplies of milk collected during one week in April. These products were dried by the Grey-Jensen process. The purified casein used in the experimental rations

was prepared by the method of Ringrose et al (1931).*

RESULTS AND DISCUSSION

The experimental results in Table 2 show the effect of the three sources of flavin on the growth of these chicks. The variation in mean body weight within groups was comparatively low. The mortality was not excessive for this experimental technic, and was due principally to the mechanical encumbrance of chicks suffering with severe cases of nutritional paralysis. The occurrence of this paralysis was general among all groups except those receiving 10 percent dried whey.

The data reproduced in graphical form were developed by establishing the line of best fit between the mean weights of the groups receiving the rations containing 0, 3, and 10 percent dried whey. The average weight of the negative control groups was taken as the zero point, and the grams gain over the weight of the negative control groups was attributed to the flavin content of the added dairy by-product supplement. By projecting on this line of best fit, the weight increases over the negative control induced by the other dairy by-products in the rations, the values of these products were interpolated as equivalents of dried whey.

The results obtained with these products

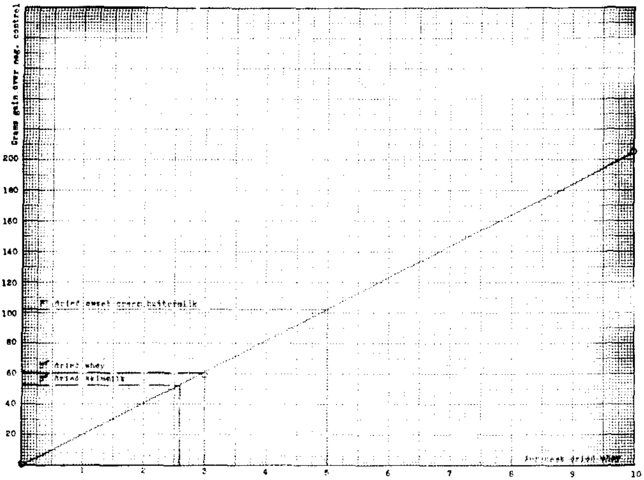

FIG. 1. Gains in weight of groups receiving dried whey over the negative control and the interpolation of the flavin content of dried skimmilk and dried buttermilk in equivalents of dried whey.

* We are indebted to Dr. N. S. Golding, Associate Dairy Husbandman, for supervising the preparation of the dairy by-products and the purified casein used in this work and for the analytical work involved.

show that dried whey was a relatively rich source of flavin when compared with dried skimmilk, yet the relative flavin value obtained was somewhat below that previously reported by Heiman, loc. cit., on products prepared by other methods.

The sample of dried sweet cream buttermilk used in this work had a flavin content almost twice as great as the dried skimmilk and 60 to 70 percent more than dried whey. Substantially the same observation has previously been made in unpublished work.

No explanation is offered for the increased flavin content in dried buttermilk. This experiment indicates, however, the need for additional information on the distribution and variability of the flavin content of dairy by-products.

SUMMARY

1. The application of a technic using replicate groups of standardized chicks of the same sex in growth studies, was demonstrated for determining the flavin (vitamin G) content of dairy by-products.

2. The neutralized dried whey used in this experiment contained between 10 and 20 percent more vitamin G than the dried skimmilk.

3. This sample of dried sweet cream buttermilk shows a flavin content 90 to 100 percent greater than the dried skimmilk and between 60 and 70 percent greater than the neutralized dried whey used in this work.

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References

Bethke, R. M., P. R. Record, and D. C. Kennard, 1933. Relation of the vitamin-G complex to hatchability and nutritive value of eggs. Poul. Sci. 12:332-3.

Davis, H. J. and L. C. Norris, 1936. The effect of process of manufacture on the vitamin-G content of dried skimmilk. Jour. Dairy Sci. 19:1-10.

Hauge, S. M. and C. W. Carrick, 1936. A differentiation between the water-soluble growth-promoting and antineuritic substances. Jour. Bio. Chem. 69:403-13.

Heiman, Victor, 1935. The relative vitamin-G content of dried whey and dried skimmilk. Poul. Sci. 14:137-46.

Norris, L. C., G. F. Heuser, A. T. Ringrose, H. S. Wilgus, Jr., and Victor Heiman, 1933. The vitamin-G requirement of poultry. Report of the Fifth World's Poultry Congress, Rome, Italy, Section 2a, No. 40.

Norris, L. C., H. S. Wilgus, Jr., A. T. Ringrose, Victor Heiman, and G. F. Heuser, 1936. The vitamin-G requirement of poultry. Bulletin 660, Cornell University Agricultural Experiment Station.

Ringrose, A. T., L. C. Norris and G. F. Heuser, 1931. The occurrence of a pellagra-like syndrome in chicks. Poul. Sci. 10:166-177.

Snedecor, G. W., 1934. Calculation and interpretation of analysis of variance and covariance. Collegiate Press, Inc., Ames, Iowa.

Wilgus, H. S., Jr., L. C. Norris, and G. F. Heuser, 1935. The relative protein efficiency and the relative vitamin-G content of common protein supplements used in poultry rations. Jour. Agr. Research 51:383-99.

The Comparative Efficiency of Vitamin D from Cod Liver Oil and Irradiated Cholesterol for Laying Birds*

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Waddell (1934) made a noteworthy contribution when he demonstrated that irradiated crude cholesterol contained a vitamin D which was as effective as the vitamin D from cod liver oil for calcification in the chick. This observation was subsequently confirmed by Bethke, Record, and Wilder (1935), Haman and Steenbock (1935, 1936), and Black and Sassaman (1936). The present study was undertaken to extend our knowledge concerning the comparative effectiveness of vitamin D from irradiated cholesterol and cod liver oil for the laying bird.

EXPERIMENTAL

Seven groups of 40 range-reared White Leghorn pullets each were confined in the same house from which all direct sunlight was excluded. Trap-nest records of egg production and monthly weights of individual birds were kept. Morning lights were used in the pens from the start of the experiment until April 15. Two male birds were placed in each pen two weeks before eggs were saved for incubation and rotated among the pens at weekly intervals.

The same all-mash basal ration, composed of ground yellow corn, 45.0; wheat middlings, 15.0; wheat bran, 10.0; ground oats, 10.0; dehydrated alfalfa leaf meal, 5.0; meat scraps, 7.5; dried skimmilk 5.0; salt, 0.5; and oyster shells, 2.0 was fed all groups, which also had free access to oyster shells and granite grit. To this ration was added 1 percent of corn oil, which supplied the following U.S.P. units of vitamin D per 100 grams of ration:

Pen 32—corn oil (negative control).
Pen 31— 25 units from irradiated cholesterol.
Pen 30— 50 units from irradiated cholesterol.
Pen 29—100 units from irradiated cholesterol.
Pen 28— 25 units from cod liver oil.
Pen 27— 50 units from cod liver oil.
Pen 26—100 units from cod liver oil.

The feed for each pen was mixed fresh every 14 to 21 days. The cod liver oil represented a concentrate containing 8,400 U.S.P. units of vitamin D per gram. The irradiated cholesterol preparation was supplied by Dr. J. Waddell. Both products were rat-assayed in our laboratory in comparison with U.S.P. Reference cod liver oil and fed on the basis of the results of these assays. The experiment was started December 5, 1935, and terminated July 7, 1936.

From February 1 to April 2 and from May 1 to May 21 all eggs available for incubation were set at weekly intervals in the same incubator. To obtain information on the effect of vitamin D on egg shell texture, eggs from 15 to 24 individual birds

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Table 1.—*Body weight, egg production, and mortality*

Pen no.	Units of vitamin D per 100 gm. of ration	Body weight		*Egg production per bird	Mortality
		initial	final		
		gm.	gm.	no.	pct.
32	None, control	1620	1615	73.6	40.0
31	25 as irradiated cholesterol	1590	1585	123.8	40.0
30	50 as irradiated cholesterol	1635	1665	126.6	42.5
29	100 as irradiated cholesterol	1605	1635	133.2	32.5
28	25 as cod liver oil	1560	1640	123.1	35.0
27	50 as cod liver oil	1640	1635	132.8	37.5
26	100 as cod liver oil	1540	1585	124.9	30.0

* Expressed on a hen-day basis.

in each pen were used for the determination of the percentage of dry shell. The eggs were collected at the beginning of each month, starting with January. To determine the percentage of egg shell, the eggs were weighed as collected, the shells removed according to the usual procedure, and washed with distilled water, to remove any adhering white, and then dried to constant weight in an electric oven. The percentage of shell is expressed on the fresh weight of the eggs.

To obtain data on the storage of vitamin D in the chicks hatched from the different pens of birds, chicks from the March 19 setting (104 days after start of experiment) were placed in wire-floor equipped brooders and fed the same rachitic ration, with distilled water to drink. The A.O.A.C. ration of ground yellow corn, 59.0; wheat middlings, 25.0; domestic casein, 12.0; dried yeast, 1.0; calcium phosphate, 1.0; calcium carbonate, 1.0; and iodized salt, 1.0 was used. At two and at four weeks the tibiae from 10 representative chicks, unless otherwise indicated, from each group were removed for ash analyses. The bone ash values are expressed on a moisture-free and fat-free basis.

As a further check on the transfer of vitamin D to the egg, line-test assays were conducted on eggs produced by pens 26, 28, 29, 31, and 32, according to the accepted 10-day procedure. The eggs for this purpose were collected from March 25 to 27. The egg yolks from 12 to 15 individual hens in each pen were used in the biological assay. The composite samples of yolks were thoroughly mixed with a weighed amount of distilled water, to facilitate feeding. Measured amounts of the diluted yolks were fed daily for the first three days of the 10-day test period in comparison with the U.S.P. Reference cod liver oil.

Table 2.—*The percentage of dry egg shell based on fresh egg weight*

Pen no.	Units of vitamin D per 100 gm. of ration	Jan.†	Feb.	March	April	May	June*	July*
32	None, control	9.48	8.83	8.42	8.09	6.29	6.39*	6.19*
31	25 as irradiated cholesterol	10.11	9.77	9.63	9.17	9.20	8.84	8.85
30	50 as irradiated cholesterol	9.95	9.45	9.04	9.27	9.01	8.97	8.59
29	100 as irradiated cholesterol	9.54	9.43	9.14	9.03	9.11	8.93	8.68
28	25 as cod liver oil	9.65	9.59	9.07	9.33	9.13	9.02	9.01
27	50 as cod liver oil	9.82	9.61	9.31	9.23	9.14	8.99	9.08
26	100 as cod liver oil	10.02	9.72	9.52	9.43	9.10	9.09	8.83

† Egg shells not thoroughly washed.
* Results based on four and five eggs, respectively.

TABLE 3.—*The comparative efficacy of vitamin D from cod liver oil and irradiated cholesterol for hatchability*

Pen no.	Vitamin D supplement per 100 grams ration	Total eggs set	Fertility	Dead germs			Fertile eggs hatched
				7 days	14 days	21 days	
			pct.	pct.	pct.	pct.	pct.
32	None, control	680	86.9	10.0	20.4	25.2	44.3
31	25 units as irradiated cholesterol	1496	87.1	7.1	5.4	14.3	73.1
30	50 units as irradiated cholesterol	1539	86.6	4.6	2.8	13.5	79.1
29	100 units as irradiated cholesterol	1667	88.2	5.0	1.9	12.2	80.9
28	25 units as cod liver oil	1459	92.9	5.4	3.8	18.1	72.7
27	50 units as cod liver oil	1757	91.2	3.4	1.8	15.9	78.9
26	100 units as cod liver oil	1600	95.3	6.1	1.2	10.7	81.9

RESULTS

The egg production, body weight, and mortality data are recorded in Table 1. It will be noted that body weight and mortality were not affected by the amount or source of vitamin D in the ration. The high mortality in all of the pens was due chiefly to fowl paralysis and closely allied disorders. The beneficial effect of vitamin D on egg production is apparent. However, there was no significant difference between the pens receiving comparable amounts of vitamin D from either cod liver oil or irradiated cholesterol—indicating that both sources of the vitamin are equally effective for egg production.

The results of the egg shell studies presented in Table 2 show the progressive decrease in the percentage of shell in case of vitamin D deficiency (pen 32) and the comparable effectiveness of the antirachitic vitamin from cod liver oil and irradiated cholesterol in the production of good egg shells.

The hatchability data recorded in Table 3 clearly show the effect of vitamin D deficiency on embryonic development. The results also show that the ration of the breeding hen must contain 50 or more U.S.P. units of vitamin D per 100 grams of total ration to produce eggs of maximum hatchability. This observation is in accord with the findings of Murphy, Hunter, and Knandel (1936b). No significant difference was noted in the hatchability of the eggs from the pens that received comparable amounts of vitamin D. This offers further evidence that the vitamin D from cod liver oil and irradiated cholesterol are equally effective for the chicken.

The results of the experiment on the storage of vitamin D in the chicks hatched from the different pens of hens are shown in Table 4. Using the percentage of bone ash in the tibiae as a criterion of the storage of vitamin D, it is apparent that the amount of this vitamin stored in the hatched chick

TABLE 4.—*Vitamin D storage in chicks*

Pen no.	Units of vitamin D per 100 gm. of ration	Chicks started	Initial weight	Two weeks		Four weeks	
				Weight	Bone ash	Weight	Bone ash
		no.	gm.	gm.	pct.	gm.	pct.
32	None, control	20	36.8	60.7	30.2±0.34	*	
31	25 as irradiated cholesterol	22	36.1	77.6	33.0±0.32	104.6	29.8±0.59
30	50 as irradiated cholesterol	21	35.4	85.4	36.0±0.53	124.2	33.1±0.43
29	100 as irradiated cholesterol	22	35.5	87.0	38.3±0.57	125.0	33.7±0.34
28	25 as cod liver oil	21	37.2	84.6	32.8±0.72	114.3	32.8±0.60
27	50 as cod liver oil	22	36.7	88.2	33.9±0.43	114.8	33.1±0.59
26	100 as cod liver oil	22	36.4	91.7	37.6±0.78	128.2	35.2±0.52

* Only three chicks survived.

TABLE 5.—*The vitamin D potency of the egg yolks*

Units of vitamin D in 100 gm. of ration fed hens	Egg yolk fed	No. of rats	Average degree of healing	*Units of vitamin D per yolk	Units of vitamin D in 100 gm. of ration fed hens	Egg yolk fed	No. of rats	Average degree of healing	*Units of vitamin D per yolk
	gm.					gm.			
None	6.0	15	0.80	5	3 units Reference cod liver oil				
	9.0	10	1.15				31	1.12	
25 as cod liver oil	4.5	8	0.75	6–7	25 as irradiated cholesterol	4.5	9	0.80	6–7
	6.0	9	0.95			6.0	10	0.92	
100 as cod liver oil	2.0	20	0.74	18	100 as irradiated cholesterol	2.0	22	0.84	18
	3.0	22	1.32			3.0	19	1.35	

* On the basis of a 15 gram yolk.

is proportional to the amount of vitamin D fed the breeding bird. These observations are in accord with the report of Murphy, Hunter, and Knandel (1936a), and Bethke and associates (1936), and contrary to the findings of Kline, Elvehjem, and Halpin (1935). It will be noted that the bone ash values at two weeks were more clear-cut in this respect than the four-week results. Similar observations have been noted by Murphy, Hunter, and Knandel (1936a). The bone ash values also suggest that the vitamin D stored in the chick was the same when equivalent rat units from either cod liver oil or irradiated cholesterol were fed to the parent stock. Further confirmation of this finding is found in the results (Table 5) of the vitamin D rat assays on the egg yolks.

SUMMARY

Studies with laying pullets on the same ration supplemented with equivalent but an increasing number of rat units of vitamin D from cod liver oil or irradiated cholesterol showed that both sources of vitamin D are equally effective for laying birds.

The results also showed that the laying bird requires 50 or more U.S.P. units of vitamin D per 100 grams of total ration for optimum hatchability.

LITERATURE CITED

1. Bethke, R. M., P. R. Record, and O. H. M. Wilder, 1935. Further studies pertaining to provitamin D of plant and animal sources. J. Biol. Chem., 112:231-238.
2. Bethke, R. M., P. R. Record, O. H. M. Wilder, and C. H. Kick, 1936. Effect of different sources of vitamin D on the laying bird, II. Storage of vitamin D in the egg and chick and mineral composition of the mature embryo. Poul. Sci., 15:336; 344.
3. Black, A., and H. L. Sassaman, 1936. The comparative antirachitic activity of several fish liver oils and other sources of vitamin D for the chicken and the rat. Amer. J. Pharmacy, 108: 237-243.
4. Haman, R. W., and H. Steenbock, 1935. The differential antirachitic activity of vitamin D milks. J. Nutrition, 10:653-666.
5. ———, 1936. The antirachitic effectiveness of vitamin D from various sources. J. Biol. Chem., 114:505-514.
6. Kline, B. E., C. A. Elvehjem, and J. G. Halpin, 1935. The effect of the hen's diet on the bone changes in rachitic chicks. Poul. Sci., 14:116-118.
7. Murphy, R. R., J. E. Hunter, and H. C. Knandel, 1936a. The effect of vitamin D intake of the hen on the bone calcification of the chick. Poul. Sci., 15:284-289.
8. ———, 1936b. The vitamin D requirements of growing chicks and laying hens. Penn. Sta. Bull., 334.
9. Waddell, J., 1934. The provitamin D of cholesterol I. The antirachitic efficacy of irradiated cholesterol. J. Biol. Chem., 105:711-739.

Agglutinin Content of Eggs from Salmonella Pullorum Infected Hens

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BEAUDETTE (1), in 1923, demonstrated the presence of specific agglutinins in the albumin of eggs from *B. pullorum* infected hens. Agglutination was most marked in 1:8 dilution of the egg albumin but occurred in some cases in a dilution of 1:64. No correlation of the blood serum and egg albumin titers was reported.

May (3), in 1924, using hens inoculated with cultures of *S. pullorum*, *P. avicida* and *S. gallinarum* found specific agglutinins in the blood serum of all birds except those inoculated with *P. avicida*. The agglutinin titer of albumin from eggs laid by hens inoculated with *S. pullorum* and *S. gallinarum* was lower than their respective blood titers.

Paille (4), in 1935, reported that the agglutinin titer of yolk of eggs from the hens infected with fowl typhoid was practically the same as that of their blood serum or the whole blood. Agglutinins were not demonstrated in the whites of these eggs.

Jansen (2), in 1936, reported that blood of hens infected with *S. enteritidis* var. essen and *S. typhi-murium*, which gave a strongly positive reaction, showed similar reactions with yolk material, but when the whole blood gave a weak reaction, negative results were obtained with the yolk. He also reports the presence of agglutinins in the yolks of eggs from hens infected with *S. pullorum*.

Studies were undertaken to determine the constancy of agglutinins in eggs produced by *S. pullorum* infected hens.

The stained-antigen and technic of the whole-blood test as recommended by Schaeffer, MacDonald, Hall, and Bunyea (5) were used. A standard loop of egg yolk or albumin was substituted for that of whole blood.

TEST NO. 1

Two 18-month-old White Rock pullorum-disease reacting hens were used in this test. Each bird gave a positive reaction to the whole-blood test. The agglutinin titer as demonstrated by the tube test was partial at 1:800 for one hen and partial at 1:400 for the other. These birds were not trap-nested, but were under observation over a period of about five months, during which time 32 eggs were layed and tested. Positive reactions were obtained with the yolks and negative reactions with the albumin of each egg. Discolored ova obtained from these hens on autopsy were cultured for *S. pullorum;* the apparently normal ova were tested for *S. pullorum* agglutinins. These organisms and agglutinins were demonstrated in material from each hen.

TEST NO. 2

Nineteen hens positive to the whole-blood test were tested by the tube method and showed titers ranging from incomplete at 1:50 to partial at 1:1600. These birds were

trapnested over a period of seven and one-half months. A total of 463 eggs were tested. The results of these tests are given in Table 1.

The hens were autopsied at the termination of the experiment and any discolored ova observed were cultured. Isolated organisms which culturally and morphologically

TABLE 1.—*Agglutination reactions of whites and yolks of eggs from pullorum-reactor hens*

Hen no.	Agglutination of blood		Total eggs tested	Agglutination of yolks					Recovery of salmonella pullorum from ova	Agglutination of immature ova
	Tube test at beginning of experiment	Whole blood test at time of autopsy		C	P	I	T	N		
1	50 P*	C	32	7	14	7	4		+	C
2	100 P	C	15	11	1	3			+	C
3	200 I	C	23	10	12	1			+	P
4	200 C	C	9	3	2	3	1		+	C
5	800 P	C	50	49	1				+	C
6	200 I	C	15	7	7	1			+	C
7	800 C	C	17	11	5	1			+	C
8	200 I	C	47	41	5	1			+	C
9	1600 P	C	45	45					+	C
10	400 C	C	27	27					+	C
11	100 P	C·	22	16	6				+	C
12	100 P	C	8	3	5				+	P
13	100 P	C	6	6					+	C
14	50 P	C	23	9	4	7	3		—	P
15	400 I	C	29	29					+	C
16	800 I	C	20	20					+	C
17	200 I	C	16	16					+	C
18	1600 I	C	34	31			2	1	+	C
19	400 P	C	25		1	23	1		+	C

* C—Complete agglutination; I—Incomplete=75 percent agglutination; P—Partial=50 percent agglutination; T—Trace=25 percent agglutination; N—No agglutination.

The yolks of 341 eggs gave complete, 63 incomplete, 49 partial, and 10 a trace of agglutination. Agglutination was not observed with the albumin of any of these eggs.

The clumps of organisms were larger in rapidly agglutinating reactions than in those agglutinating slowly. Usually when the yolk agglutination occurred rapidly with comparatively large agglutination particles the blood titer of the respective bird was high. One exception was noted; none of the 25 eggs layed by a Barred Rock hen whose blood titer was incomplete at 1:400, gave complete reactions.

Yolks of 92 eggs layed by these hens were cultured for *S. pullorum* prior to their use in the agglutination test. All media inoculated with this yolk material remained sterile.

resembled *S. pullorum* were used as antigen for agglutination tests with known positive and negative pullorum sera. Judged by this criterion *S. pullorum* was recovered from 18 of the 19 hens. The hen from which *S. pullorum* was not recovered had a blood titer incomplete at 1:50. In tests of 23 eggs which she produced, nine gave complete, four incomplete, and seven partial agglutinations while three showed only a trace of agglutinations.

Yolk material, collected at the time of autopsy of the hens, from apparently normal but incompletely developed ova gave positive agglutination reactions in 16 hens and incomplete in three. One of the latter was the hen from which *S. pullorum* was not isolated.

SUMMARY

Yolk and albumin from 495 eggs layed

by 21 hens having *S. pullorum* titers of their blood serum ranging from incomplete at 1:50 to partial at 1:1600 were tested by the stained-antigen-plate method. Agglutinins were demonstrated in the yolk of each egg but in no instance in the albumin.

Complete agglutination of the antigen occurred regularly with yolk material from eggs produced by six of these hens. Eggs from the other hens gave reactions that varied from complete to a mere trace of agglutination.

Ninety-two of these eggs, the total number layed during October, November, and December, were cultured for *S. pullorum* with negative results.

Blood collected from each of the 21 hens at the time of autopsy gave complete agglutinations in the whole-blood-plate tests.

S. pullorum cultures were obtained from discolored ova of 20 of the 21 hens.

Material obtained at the time of autopsy from apparently normal but incompletely developed ova gave complete agglutination with yolk from 18 of the hens and incomplete with that from three.

Agglutination of the antigen usually was most rapid and the clumps largest with material from the high-titer birds.

REFERENCES

1. Beaudette, F. R. 1923. *Agglutinins for Bacterium Pullorum in Hen's Eggs.* Jour. Immunology, 8:493–499.
2. Jansen, J. 1936. *Agglutination of Yolk Substance with Salmonella of Ducks.* Dtsch. tierarztl. Wschr. 19:340-341.
3. May, H. G. 1924. The Examination of Eggs from Infected and Immunized Hens with Germicidal Tests on Albumin and Blood Serum. Bull. 197. Agr. Expt. Sta. of Rhode Island State.
4. Paille, R. 1935. Avian Typhoid and the Ability of Eggs to Agglutinate. Rev. Vét. et Jour. de Méd. Vét., 87:578.
5. Schaeffer, J. M., A. D. MacDonald, W. J. Hall, H. Bunyea, 1931. A Stained Antigen for the Rapid Whole Blood Test for Pullorum Disease. Jour. Am. Vet. Med. Ass., 79:236-240.

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